Universidade de Aveiro Departamento de Biologia
2008

Joaquim Alexandre Macedo de Sousa

Novos instrumentos para a comunicação e avaliação de risco de vectores deletérios para a biodiversidade

New tools for the communication and risk assessment of vectors influencing biodiversity

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Prof. Doutor Amadeu Soares, Professor Catedrático do Departamento de Biologia da Universidade de Aveiro e co-orientação do Doutor José Vicente Tarazona, Coordenador da Área de Ecotoxicologia e Avaliação de Riscos Ambientais do Instituto Nacional Espanhol de Investigação e Tecnologia Agrária e Alimentar (INIA).

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palavras-chave

avaliação de risco, métodos probabilísticos, herbicidas, glifosato, lamas de etar, cádmio, LAS, biodiversidade, rede natura 2000, agricultura, comunicação de risco.

resumo

Em Portugal, mais de 25 % dos habitats da Rede Natura 2000 dependem da manutenção de práticas associadas à agricultura extensiva, sendo o valor médio da UE-15 de 18 %. No seguimento dos objectivos da Rede Natura 2000, os Estados-membros apresentaram uma lista de sítios de importância comunitária (SIC) e de zonas de protecção especial (ZPE) de aves, com requisitos especiais em termos de gestão e restrições às actividades desenvolvidas dentro e nas suas fronteiras. De acordo com o Instituto de Conservação da Natureza a ZPE de Castro Verde, no Alentejo, é a área mais importante a nível nacional para a conservação de aves estepárias, como a Abetarda (*Otis tarda*), o Tartaranhão-caçador (*Circus pygargus*) ou o Peneireiro-das-torres (*Falco naumanni*). A principal unidade paisagística desta ZPE é caracterizada por um mosaico de campos de cultivo extensivo de cereais, restolho, terrenos lavrados e terrenos em pousio, estes últimos utilizados normalmente como pastagem de ovelhas. As principais ameaças à ZPE estão identificadas e resultam do processo dual de abandono dos terrenos agrícolas menos férteis e intensificação da agricultura nos restantes. Com o objectivo de avaliar os efeitos da agricultura tradicional e da sua intensificação, nomeadamente o aumento da utilização de herbicidas e aplicação de lamas de etar usadas como fertilizantes, foi desenvolvido um modelo conceptual e avaliado com a abordagem probabilística. Este modelo inovador foi utilizado numa avaliação de risco de alto nível onde foram tidos em consideração os parâmetros biológicos das populações de aves desta área protegida. Considerou-se um cenário em que a transferência de químicos ocorre maioritariamente ao longo da cadeia trófica, de acordo com os diferentes comportamentos alimentares das diferentes espécies, mas também dentro da mesma espécie quando se verificam hábitos alimentares diferentes (e.g. adultos e juvenis). Foi também estudada, através de um inquérito, a percepção aos riscos na ZPE de Castro Verde de diferentes intervenientes, de forma a contribuir para o desenvolvimento de um directório para comunicação do risco da agricultura tradicional num sítio Natura 2000.

keywords

risk assessment, probabilistic methods, herbicides, glyphosate, sewage sludge, cadmium, LAS, biodiversity, natura 2000 network, agriculture, risk communication.

abstract

In Portugal more than 25 % of Natura 2000 Network habitats depend upon the continuation of extensive farming practices whereas the average EU-15 value in 2004 was of 18 %. Following the Natura 2000 Network objectives, Member States have proposed a list of sites of communal interest (PSCIs) and special protection areas (SPA) for birds, with specific management requirements and necessary restrictions on activities carried out within and around their borders. According to the Portuguese Institute for Nature Conservation the SPA of Castro Verde in southern Portugal, Alentejo, is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*), the Montagu's Harrier (*Circus pygargus*) or the Lesser Kestrel (*Falco naumanni*). The main landscape of this SPA is characterized by a mosaic of extensive cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep. The main threats to the SPA of Castro Verde are identified and result from the dual process of the abandonment of the less fertile agricultural soils with the intensification of agriculture in the remaining land. In order to assess the effects of traditional agriculture and its intensification, namely the increase in the input of herbicides and sewage sludge used as fertilizer, a conceptual model was developed and assed with a probabilistic approach. This innovative model was used in a high tier risk assessment by taking into account the biotic parameters of bird populations from this protected area. The transfer of chemicals was considered to occur mainly through a realistic trophic chain scenario according to the different feeding behaviour among different species and even within the same species when having different feeding habits (e.g. adults and juveniles). The perception of risks to the SPA of Castro Verde to different stakeholders was also studied with a questionnaire-based survey thus contributing for the development of a risk communication framework for risks posed by extensive agriculture in a Natura 2000 Network site.

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Thesis Structure

The present thesis will be structured in six chapters. In the first chapter, the basic principles and concepts underpinning this work will be drawn in a General Introduction followed by the description of the Objectives with the rationale and scope of the thesis. In chapters two to five the work will be described in detail in the form of four Manuscripts that will be later on submitted to relevant SCI journals. Finally the major achievements of the thesis will be discussed in the General Discussion chapter.

CHAPTER 1

Chapter 1. General Introduction and Objectives

Biodiversity in Europe

One of the outcomes of the UN Earth Summit held at Rio de Janeiro (Brazil) in 1992 was the Convention on Biological Diversity (CBD) [1], where "biological diversity" was defined as *the variability among living organisms from all sources including, among others, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems*. The services healthy ecosystems with high biodiversity may deliver to mankind, often at no cost, have been pointed out by the Millennium Ecosystem Assessment (MA) [2] namely production of food, fuel, fibre and medicines, regulation of water, air and climate, maintenance of soil fertility, or cycling of nutrients. But MA has also shown that in recent times, especially over the past fifty years, the decline of biodiversity and respective changes in ecosystem services have been taking place, mostly due to habitat change, climate change, invasive alien species, overexploitation, and pollution. Needless to say human activities have been increasingly accentuating these deleterious drivers. Moreover changes in species diversity affect the ability of ecosystems to recover from disturbances, and thus underpin the resilience of ecosystems as well the services they provide [3].

According to the World Conservation Union (IUCN) [4], 147 vertebrate (mammals, birds, reptiles, amphibians and fish) and 310 invertebrate (crustaceans, insects and molluscs) species that occur in Europe are considered to be globally threatened, therefore categorised as critically endangered, endangered, or vulnerable. Albeit these numbers there were several significant commitments made by the EU regarding biodiversity along with many protection policies as part of the European strategy to conserve its critical wildlife and habitats. One important milestone was the signature of the above mentioned UN CBD where countries from the UN, EU countries included, recognized the biodiversity loss and its significance to society. In 1998 the EC Biodiversity Conservation Strategy was launched providing a comprehensive response to the many requirements of the CBD. Later on in 2001 the EU Heads of State or Government agreed to halt the decline of biodiversity by 2010 in the Göteborg European Council. One year later 130 world leaders, including the EU's, agreed to significantly reduce the rate of biodiversity loss by 2010 in the Plan of Implementation from the Johannesburg 2002 World Summit for Sustainable Development. At an important stakeholder conference held under the Irish Presidency of the European Council in Malahide in 2004, a broad consensus was achieved on priority objectives and a set of biodiversity indicators towards meeting the 2010 commitments, expressed in the "Message from Malahide". These biodiversity indicators were based on the first set of indicators adopted globally earlier in 2004 at the CBD 7th Conference of the Parties in Kuala Lumpur. By 2005 EU established a Streamlining European 2010 Biodiversity Indicators, where 26 indicators were proposed for different focal areas of biodiversity, including public awareness. Recently, in 2006 with an EC Communication on halting the loss of biodiversity, the extent of biodiversity loss was outlined but the adequacy of the EU response so far was also reviewed. In respect to the EU Biodiversity policy the basis for action is provided by the Birds and the Habitats Directives (the so-called "nature directives"). The strategic framework for the Commission's environmental policy is set by the Environment Action Programmes of the EC. The Sixth Action Programme for 2002-2012 [5] frames "Nature and Biodiversity" with the other environment priority areas and promotes full integration of environmental protection requirements into all Community policies and actions and provides the environmental component of the Community's strategy for sustainable development.

The Mediterranean Ecoregion

The highest number of plant and animal species in Europe is hosted in the Mediterranean basin, which has been identified by Conservation International as one of the world's 34 biodiversity hotspots [6]. The Mediterranean Hotspot surrounds the Mediterranean Sea and stretches west to east from Portugal to Jordan and north to south from northern Italy to Morocco, also including parts of Spain, France, the Balkan states, Greece, Turkey, Syria, Lebanon, Israel, Egypt, Libya, Tunisia and Algeria, with a total extent of more than two million square kilometres (figure 1.1). Islands from the Mediterranean Sea and from the Atlantic Sea – the Macaronesian Islands of the Canaries, Madeira, the Selvages (Selvagens), the Azores, and Cape Verde – are also part of this hotspot [6]. Physical background diversity is settled by numerous mountains as high as

4000 meters, peninsulas, islands and archipelagos. The bimodal weather pattern, that provides the unity to this ecoregion, is dominated by hot, dry summers, and cool, wet winters, with average annual rainfall ranging from less than 100 millimetres in desert territories to more than 4000 millimetres on certain costal massifs. In the western Mediterranean, the Iberian Peninsula, for at least two months each year there is frequently no precipitation al all, and most plants and animals experience a water deficit thus having developed ecophysiological or behavioural adaptations [7]. All this variety contributes to a high proportion of ecologically valuable areas and exceptional concentrations of biodiversity with 22500 species of vascular plants, nearly 500 bird species, more than 220 terrestrial mammals, more than 225 reptile species and nearly 80 amphibians, of which can be counted, respectively, 11700, 25, 25, 80, and 30 endemic species [6].

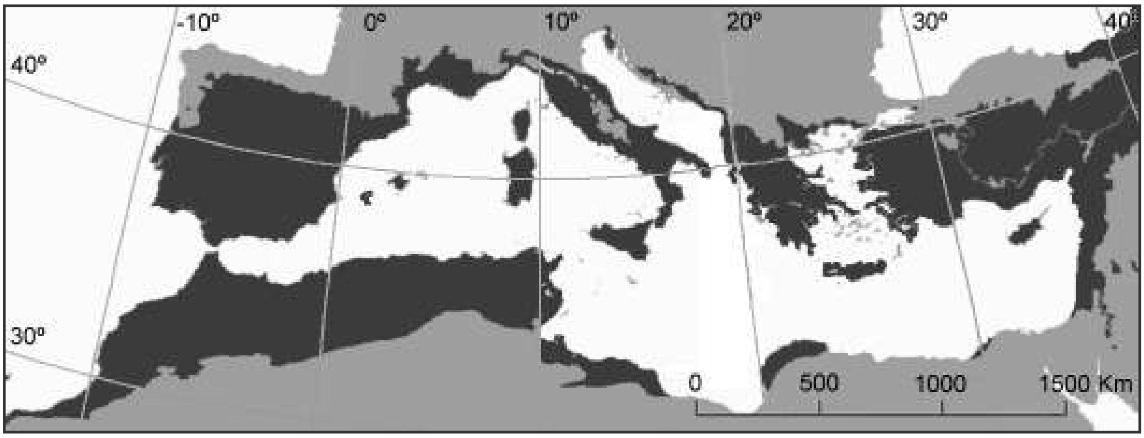

Figure 1.1. The Mediterranean Basin Hotspot. The dark shadows indicate the hotspot regions. Although Macaronesian Islands of the Canaries, Madeira, the Selvages (Selvagens), the Azores, and Cape Verde are not included in the map, they are part of the hotspot.
Adapted from the European Environment Agency Maps and Graphs data service [8] with information from the Biodiversity Hotspots webpage [6].

Agriculture and Biodiversity

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. Less than a fifth of the European land can be regarded as not directly managed. And of course biodiversity includes both managed and unmanaged ecosystems. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [9].

Following the overall trend, biodiversity in Europe's farmland has declined strongly in the last decades with a special emphasis to bird populations [10]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [11]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems. On the other hand the intensification of agriculture and concomitant increase in nutrient and pesticide inputs (chemical inputs will be further discussed in this chapter), generally leads to the decrease of biodiversity. Another factor that may jeopardise biodiversity of HNV farmland is agriculture abandonment as the result of low productivity that drive the socio-economic conditions of in rural areas unfavourable [12]. The Common Agriculture Policy (CAP) whilst being considered responsible for loss of biodiversity in rural areas by supporting greater productivity and consequently leading to agriculture intensification the [13], its agri-environment schemes – that exist since 1992 but became compulsory since the 2003 CAP reform – are important as funding instruments for promoting pro-diversity measures [12]. Therefore agriculture may be looked at not only for food production but also in the perspective of providing environmental services.

Protected Areas in Europe

Protected areas are fundamental policy tools for biodiversity and ecosystems conservation, especially for sensitive habitats [2].

The IUCN defines 6 categories of protected areas, depending on the management objectives, that are implemented in a network of 83 States [14]: Ia Strict Nature Reserve, Ib Wilderness Area, II National Park, III Natural Monument, IV Habitat/Species Management Area, V Protected Landscape/Seascape, and VI Managed Resource Protected Area. But at the EU level a network of protected areas, Natura 2000 Network, is being built on the designation of areas for conservation under the EU Birds and Habitats directives. Endangered and rare birds at the European or global level were firstly addressed by the Birds Directive [15], but this piece of legislation was afterwards complemented by the Habitats Directive [16] where habitats and other wildlife species were also considered. Thus Member States have designated Special Protected Areas (SPAs) for wild birds and then proposed Sites of Community Interest (SCIs) for habitats and endangered species, that encompass the Natura 2000 Network. In December 2006 it already covered more than 20 % of EU-25 territory [17]. Once in Natura 2000 Network the conservation status of habitats and species listed in the nature directives must to be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [15; 16].

SPA of Castro Verde

In Portugal more than 25 % of Natura 2000 habitats depend upon the continuation of extensive farming practices – that sustain HNV farmland – whereas the average EU-15 value in 2004 was of 18 % [11].

Figure 1.2. Municipalities within the SPA of Castro Verde. Adapted from the Natural Patrimony Information System of the Portuguese Institute for Nature Conservation [19].

Steppic areas are typical examples of HNV farmland from southern Europe with bird assemblages of conservation concern [12]. According to the Portuguese Institute for Nature

Conservation [18] the SPA of Castro Verde (figure 1.2) in southern Portugal, Alentejo, is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu's Harrier (*Circus pygargus*).

Classified under national law by the "Decreto-Lei nº 284-B/99" in September 1999, the SPA of Castro Verde includes six municipalities of which the municipality of Castro Verde has the higher percentage of land, 55 %, out of a total area of 79007.17 ha. In the last forty years average annual temperature from this region was approximately 22 ºC and annual rainfall 500-600 mm. For the same time span, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [20].

The main habitat of this SPA is characterized by extensive farm fields with no arboreal vegetation and some less representative habitats with no agricultural use such as shrublands (of scrub *Cister ladanifer*) and woodlands (mainly holm oak *Quercus rotundifolia* but also a few olive groves *Olea europea*). The overall scheme of farming is based on the following traditional rotation system: 1st year, primary cereal (wheat *Triticum aestivum*) – 2nd year, secondary cereal (oat *Avena sativa*) – 3rd year, fallow – 4th year, fallow – 5th year, land ploughed to reinitiate the cycle [18]. Cereals are usually sown in September-November and harvested in June-July [21] and leguminous crops (e.g. chickpea *Cicer arietinum*) are also sown in smaller amounts in summer. The main changes on this scheme occur on the duration of the fallow that is dependent on the fertility of the fields. Historically, livestock farming is based on extensive sheep production but nowadays cattle production is rapidly growing [18]. All these activities result in a landscape mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [22; 23]. The origin of this extensive agricultural system lies in the agricultural rush (Wheat Campaign) that took place in the 1930s as an attempt to make the country self-sufficient in wheat production. As a result of clearing of all existing vegetation (trees included) and ploughing of all types of soil, soil erosion was accelerated and the early intensive cereal cultivation system gave place to the present extensive agricultural pattern and livestock farming [24]. This is an area with the generality of soils that are poor and unsuitable for agriculture, where 75 % of the agricultural area is in fallow or works as permanent pasture land. Thus a low intensity non-irrigated cereal farming land it represents a marginal economic system with a yield of 14 % of the EU average [25].

The main threats to the SPA of Castro Verde are identified and result from the dual process of the abandonment of the less fertile agricultural soils with the intensification of agriculture in the remaining land. The result will most certainly lead to the impoverishment of this refuge for steppic birds due to: possible forestation because of the rather advantageous EC funding; increasing in cattle production, disappearance of the traditional cereal-fallow rotation, and installation of fences and land irrigation systems [18; 25]. Furthermore an input of chemicals in the ecosystem will tend to increase as it will be explained later on. One of the ways to overcome this hazardous trend is the financial support designated in this SPA as the Zonal Program of Castro Verde (ZPCV). These agri-environmental measures' management objectives are settled in national law, annexe I of "Portaria nº 1212/2003". Although the ZPCV was implemented in 1995, it was reviewed by the referred piece of legislation in October 2003 [18]. Shortly, if allows financial compensation to farmers who voluntary agree to maintain the traditional agricultural system with the cereal-fallow rotation, in an area larger than one hectare.

Birds in Castro Verde's SPA

Farmland birds are considered indicators for biodiversity because they are dependent on the ecological structure of agricultural habitats [12; 11; 3]. Since the 1970s it has been taking place an overall decline in farmland bird populations across Europe, a declining trend that is not apparent in bird assemblages of other habitats. This long-term trend suggests that the driver factors are specific to this habitat [10] and in fact agriculture intensification may account for the decline of more than 40 % of the bird species [12]. But as evidenced by Donald *et al.* [10] not all farmland species exhibited patterns of population decline. And if in the case of Great Bustard and the Lesser Kestrel there was a negative trend of, respectively, -1.1 and -1.39, other birds like the Montagu's Harrier exhibited positive mean trends (0.64).

Great Bustard (Otis tarda)

The Great Bustard (*Otis tarda*), family *Otididae*, is one of the largest birds of Europe and one of the heaviest flying birds of the world, typical of the steppic habitats and open lands with non-intensive farming [26]. Its populations though widely distributed, from the Iberian Peninsula to eastern Asia, are generally separated and consist of a few tens to several hundred individuals [27]. In Portugal there are estimated to exist 1150 individuals 80 % of which inhabit the SPA of Castro Verde. *O. tarda* is listed in the annex I of the Birds Directive [15] as well as categorized as VULNERABLE in the 2007 IUCN Red List [28] and in the Portuguese Vertebrate Red List [29].

This species has an accentuated sexual dimorphism with males weighting around 16 kg, which is 2 to 4 times the weigh of females (3-4 kg) [30], and measuring up to 1 m being therefore ca 50 % bigger than females [28]. The Great Bustard is a gregarious bird that lives in flocks with a variable number (4-16) of individuals around the year [30]. Feeding is the most time-consuming activity for the *O. tarda* [31]. They are omnivorous birds that feed mainly upon green plant material, and arthropods and seeds to a lesser extent [26; 27]. In fact green plant material accounts for 71 % in summer and autumn, and 95 % in spring and winter [32].

Lesser Kestrel (Falco naumanni)

The migratory Lesser Kestrel (*Falco naumanni*), Family *Falconidae*, has a Mediterranean range for breeding, heading south to Africa in winter, particularly to the southern Sahara region. It forages steppic habitats and grasslands with non-intensive cultivation [33]. In spite of being considered an endangered species all over Europe [34; 35], in Portugal it has been developing an increasing trend since 2001 (289 couples) until 2006 (445 couples), being the SPA of Castro Verde the territory for 73 % of the Portuguese breeding population [36]. *F. naumanni* is listed in the annex I of the Birds Directive [15] as well as categorized as VULNERABLE in the 2007 IUCN Red List [33] and in the Portuguese Vertebrate Red List [29].

This small hawk measures ca 30 cm and seldom exceeds 200 g of weight. Its rusty plumage bears him the right camouflage for the arid habitats where it lives [36; 33]. The Lesser Kestrel is a gregarious raptor that feeds mainly upon insects and preying activity tends occur in the surroundings of the colonies [37].

Montagu's Harrier (Circus pygargus)

The Montagu's Harrier, Family *Accipitridae*, has a widespread but patchy breeding distribution in Europe, which constitutes over 50% of its global breeding range [38], and it winters sub-tropical Africa and India, and around the Mediterranean Sea [39]. Although it is originally a marsh harrier it colonized the great extent of farmland that covers Europe [40]. The Portuguese population is the third largest in Europe with 900-1200 couples, being the breeding group in the SPA of Castro Verde the largest in the country [41]. *C. pygargus* is listed in the annex I of the Birds Directive [15], and tough considered of LEAST CONCERN in the 2007 IUCN Red List [38] it is categorized as VULNERABLE in the Portuguese Vertebrate Red List [29].

Being the smallest within the Harriers, this species measures 43-47 cm [39] and weights around 345 g although males tend to increase in body size as one goes west and south in Europe [40]. It is frequent to observe the reunion of couples in colonies while breeding showing therefore gregarious behaviour [41]. The Montagu's Harrier diet includes small mammals, mainly rodents, and occasionally small birds and large insects [42].

Toxic inputs in the SPA of Castro Verde

The present agricultural and livestock activities going on and the future trends of the SPA of Castro Verde make possible the input of several chemicals in this area of conservationist concern.

Herbicides

The herbicide glyphosate is marketed as a non-selective, broad-spectrum, post-emergence herbicide and is applied in this farmland area before seedling. It is a widely popular herbicide known for its effective control of competing vegetation, rapid inactivation in soil, and supposedly low toxicity to terrestrial invertebrates and mammals [43; 44]. But in fact it has been reported to affect the survival of earthworms [45], exert hepatic toxic effects to small rodents [46], and to affect not targeted plants in adjacent habitats to cultivated fields

[47], and therefore may threaten the wildlife vertebrates that rely upon these communities as food items.

Wastewater Sludge

In recent years in Castro Verde, wastewater sludge were used as fertilizers in a program aiming to prevent desertification and soil erosion [48] but these products were not assessed for the risks to the ecosystem in spite of the performed chemical analysis. Previous studies on the risks of the use of sewage sludge as fertilizer to soil microarthropod populations under Mediterranean climatic conditions revealed an impoverishment of the community structure and decrease in the diversity of Acari [49; 50]. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [51]. Moreover, problems with disposal of the accumulating sewage sludge in the municipal plants will probably lead to a compulsory use of these products as soil fertilizers. The presence of metals in the sludge is a clear concern and is regulated by the Sewage Sludge Directive 86/278/EEC [52]. Cadmium can be used as a model metal. According to risk assessment report of Cadmium (Cd) edited by the European Chemicals Bureaus [53] sewage sludge is a minor source of Cd for soils on an average basis; but it is a major source of Cd in soils where sludge is applied. However this risk assessment report does not assess the risks of Cd on soils where sludge is applied although it describes the potential hazards of Cd to soil fauna and plants from the revision of several research papers. Recently, the concern on the presence of micropollutants in the sludge has been extended to organic chemicals [54]. A large list of chemicals used in consumer products can be found in the sludge. Detergent components are of special concern in countries such as Denmark [55]. Linear alkylbenzene sulphonates (LAS) are the most widely used anionic surfactants in cleaners and detergents and are a major organic contaminant present in sewage sludge [56]. Terrestrial animals are not likely to be affected by sewage sludge LAS [55; 57] but its repeated addition needs to be assessed [58].

Veterinary Medicinal Products

The existence of livestock grazing in the area creates a less conspicuous pathway for the input of high local concentrations of toxicants into the ecosystem due to the veterinary medicinal products that can be found in the livestock dung and urine [59]. In the case of Castro Verde, the intensification livestock farming of cattle will most certainly become a vector for this type of contamination in a near future if not already present. Veterinary medicines are important safeguarding the health and welfare of livestock [60] but may have a potential impact in terrestrial ecosystems [61-64].

Europe's chemicals policy

According to the UN Programme of Action from the Earth Summit of Rio de Janeiro 1992, Agenda 21, improved risk assessment is necessary for the safe use of toxic chemicals (Section II, Chapter 19) [65]: "*Thousand of chemicals are used in every aspect of human endeavour but the long-term health and environmental risks of most of them are unknown. 95 % chemical manufacturing involves only 1500 chemicals but crucial data for risk assessment are lacking for many of them.*" In Europe the utilization of chemicals by human activities is regulated by several pieces of legislation implemented through guidance documents that foresee risk assessment protocols as the tool to set the impact of chemical contamination on biota [66]. With the introduction of the REACH, Regulation (EC) No 1907/2006 [67], risk assessment processes for existing substances will be further regarded and hasten.

For instance, if we consider the main probable toxic inputs in the SPA of Castro Verde, herbicides and sewage sludge, an assessment of their risks may be based in appropriate European guidelines. In the Annex VI of the Directive 91/414/EEC the detailed evaluation and decision making criteria for plant protection products (e.g. herbicides) is described [68]. Additional technical guidance is presented in Guidance Documents [69; 70] and in the outputs of the recent European Food Safety Authority scientific workshop on the revision of a guidance document on assessment of pesticide risks for birds and mammals [71]. Data for the risk assessment of sewage sludge may be found in the EC Directive 86/278/EEC on the use of sewage sludge in agriculture [52], namely the limit values for heavy metals, but limits for organic compounds are not included. The limit values for compounds such as LAS can only be found in the EC Working Document on Sludge [72] on the revision of the Directive 86/278/EEC. Nonetheless methodologies for the risk assessment of metals and organic compounds are described in the EC Technical Guidance Document of 2003 [73]. Similarly, veterinary medicines are covered by Directive 2004/28/EC [74] and Regulation (EC) No 726/2004 [75]. In 2007 the European Medicines Agency launched a guideline [76] on the assessment of veterinary medicines in support of two other guidance documents on the environmental risk assessment of veterinary pharmaceuticals adopted following the international harmonisation process through the Veterinary International Conference on Harmonisation [77; 78].

(Probabilistic) Risk Assessment

Ecological Risk Assessment is a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors [79]. This scientific step underpins the decision-making process defined as Risk Management – that involves considerations of political, social, economic, and technical factors – by providing information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard [80].

The standardization for terrestrial risk assessment has been addressed at the EU level [81] with an holistic approach selecting key route-receptor interactions for each assessment as mentioned by Tarazona *et al.* [82]. In a general way risk assessment methodology is based on the systematic and tiered comparison of the exposure (predicted environmental concentration – PEC) against the effects (predicted no effect concentration – PNEC) with the application of safety factors to account for uncertainty [66]. But as Calow [83] has pointed out already 15 years ago, when looking at the challenges for ecotoxicology in Europe “this is not quite risk assessment in the sense of explicitly characterizing the probability of populations or communities becoming impaired to defined extents”. A way to handle this bias is to include ecological considerations in risk assessment [84] or by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [85]. The last is called the probabilistic risk assessment approach where instead of point estimates a distribution for exposure (exposure/environmental concentration distribution – ECD) and/or effects (species sensitivity distribution – SSD), and concomitant risks may be obtained [86]. Probabilistic methodologies have been considered valid and scientifically sound or have been putted forward by many international bodies involved in the field of risk assessment, e.g., EC [66], SETAC [87], ECETOC [88], OECD [89] or USEPA [79]. And in an European Workshop on Probabilistic Risk Assessment for Pesticides [90], the main advantages of this approach were highlighted to aquatic organisms, and terrestrial plants, vertebrates and invertebrates: helps to quantify variability and uncertainty, can produce outputs with more ecological meaning (e.g. probability and magnitude of effects), makes better use of available data, identifies most significant factors contributing to risk, can provide an alternative to field testing or helps focus on key uncertainties for further study in the field, and promotes better science by considering multiple possibilities. Moreover when considering the probabilistic methods instead of the regular deterministic approach, risk assessment is more transparent, with the sources of uncertainty identified, allowing therefore a clearer communication of risk [81].

Risk Communication

Risk Communication is defined by the OECD [80] as the interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public. Thus communication is an important tool in the understanding of environmental problems, in the orientation of decision-making and ultimately inducing a cultural change towards sustainability [91]. But, although risk communication is recognized as part of the assessment of chemicals' protocols in Europe [81; 66], in the USA [79], and at the international level, [80] it is not clear as it relates structurally to the assessment and the management phases. In the USA it is a differentiated step within risk analysis that includes risk assessment, risk management and risk communication [92], whereas in Europe it is included in the risk management process that comes after risk assessment [93]. Nonetheless the goal of risk communication is fully recognized, which is to enhance the likelihood that risk management decisions will incorporate the results of the risk assessment and that both the assessment and the decisions will be understood and accepted by potentially affected individuals or groups [94] as well as the general public. In spite of this, to our knowledge, there are not any available protocols or guidelines for communicating the risks of chemicals to the ecosystems, and most of risk communication processes are related to human health.

Objectives

The main objective of this dissertation was to assess and set the first steps for the communication of risks posed to the ecosystem from the chemical inputs due to extensive agriculture within a Mediterranean protected area.

It is perfectly clear the importance of halting the loss of biodiversity and to properly assess the utilization of chemicals in order to achieve a sustainable development in our society. But how far are these two subjects being brought together? How suitable are the available risk assessment protocols for protecting ecologically valuable areas? How is the reality towards the development and usage of tools for communicating risks?

If we look at the Streamlining European 2010 Biodiversity Indicators, amongst the 26 proposed indicators, only for indicator 19, nitrogen balance in Agriculture, environmental risk assessment is proposed as a tool for analysis of options. And when looking at the legislation for protection of biodiversity, the birds' directive or specific management plans like the Portuguese Sectorial Plan for the SPA of Castro Verde, the assessment of chemicals is disregarded albeit being mentioned the problems of the intensification of agriculture namely the increase of fertilizers and herbicides input.

Regarding European risk assessment protocols it may be observed that they are insufficient to protect ecological values in specific areas, because they only present generic models and exposure scenarios that do not cover different levels of biodiversity protection in different eco-regions. It would be important to re-evaluate chemicals being used in the protected areas, namely the Natura 2000 Network, and also to map the ecological interest of risk assessment at European level. After all what do we want to protect?

The importance of knowing the perception of people towards environmental risks that may affect biodiversity in order to develop tools for communication is fully recognized but seldom used in supporting the management of risks. Especially when dealing with ecosystems with conservationist concern it is essential to drive everyone's attention for the risks of activities independently of how little conspicuous the risks may be.

New tools for communication and risk assessment…

Bearing all the previous aspects in mind our work aimed to develop new tools for scientifically sound based environmental policy by:

a) Developing an innovative model with a specific exposure scenario in a Mediterranean area of concern in terms of biodiversity, allowing higher tier refinements based on biotic parameters for three bird species listed in annex I of the birds' directive, Great Bustard *Otis tarda*, Lesser Kestrel *Falco naumanni* and Montagu's Harrier *Circus pygargus*.
b) Using a probabilistic approach to characterize risks posed by the different chemicals that take part in the extensive agricultural activities in a Natura 2000 site, selecting examples in each group (Glyphosate as an herbicide, and Cadmium metal and LAS abundantly present in wastewater sludge) and using the frameworks proposed in the respective piece of legislation, guidance documents and/or risk assessment report;
c) Contributing for the development of a risk communication framework that takes into account the public awareness and perception of the risk, the necessity of illustrating the overall impression of the risk to farmers as the major actors in the continuation of extensive agricultural practices, and to make the risk assessment procedures part of risk management options to local authorities and decision-makers.

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CHAPTER 2

Chapter 2. A conceptual model for assessing risks in an European Mediterranean protected area

Abstract

Every year millions of tons of chemical products are disposed to the environment as a result of human activities, with deleterious consequences to biodiversity. In Europe the biodiversity policy basis for action is provided by the Birds and the Habitats Directives. According to these directives a network of protected areas is being built across EU countries encompassing the Natura 2000 Network. But the management plans of these protected areas do not require an ecotoxicological assessment of chemicals used within its limits. As for risk assessment protocols described in EC pieces of legislation and technical guidance documents, they are generic guidelines that not take into consideration regional particularities, e.g. the Mediterranean ecoregion specificities, and its local ecological values. Herewith we present a conceptual model for the assessment of risks posed by agriculture to bird species of conservationist concern from Natura 2000 Network sites; an example is set in a cereal steppe of the Iberian Peninsula. Hazards identified are related to the utilization of herbicides, disposal of sewage sludge to be used as fertilizer, and the input of veterinary pharmaceuticals that can be found in livestock dung and urine. This innovative model, to be used in high tier risk assessment, takes into account the biotic parameters of bird populations from this protected areas. The transfer of chemicals is considered to occur mainly through a realistic trophic chain scenario according to the different feeding behaviour among different species and even within the same species when having different feeding habits (e.g. adults and juveniles). Moreover, the probabilistic approach is proposed in order to perform a transparent risk assessment and clearer risk communication.

Keywords: conceptual model, probabilistic risk assessment, protected area, agriculture, herbicide, sewage sludge, veterinary medicinal product.

Introduction

According to the UN Programme of Action from the Earth Summit of Rio de Janeiro 1992, Agenda 21, improved risk assessment is necessary for the safe use of toxic chemicals (Section II, Chapter 19) [1]: "*Thousand of chemicals are used in every aspect of human endeavour but the long-term health and environmental risks of most of them are unknown. 95 % chemical manufacturing involves only 1500 chemicals but crucial data for risk assessment are lacking for many of them.*" In Europe the utilization of chemicals by human activities is regulated by several pieces of legislation implemented through guidance documents that foresee risk assessment protocols as the tool to set the impact of chemical contamination on biota [2]. With the introduction of REACH, Regulation (EC) No 1907/2006 [3], risk assessment processes for existing substances will be further regarded and hasten. But in spite of the many strides in reducing the chemical contamination and pollution its effects on human health and biodiversity are still quite evident. Maintaining the richness of European biodiversity and ecosystems is essential when considering present and future ecosystem services [4].

At the EU level a network of protected areas, Natura 2000 Network, is being built on the designation of areas for conservation under the EU Birds and Habitats directives. Endangered and rare birds at the European or global level were firstly addressed by the Birds Directive [5], but this piece of legislation was afterwards complemented by the Habitats Directive [6] where habitats and other wildlife species were also considered. Thus Member States have designated Special Protected Areas (SPAs) for wild birds and then proposed Sites of Community Interest (SCIs) for habitats and endangered species, that encompass the Natura 2000 Network. Once in Natura 2000 Network the conservation status of habitats and species listed in the nature directives must be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [5; 6].

This paper aims to present a critical review on protocols for the assessment of risks posed to Mediterranean protected areas, namely a bird SPA. Moreover herewith we present a generic approach with a site-specific conceptual model and exposure routes, that may take place due to the input of chemicals from extensive agriculture, for a cereal steppe in the Iberian Peninsula. The paper is structured as follows. A policy background is drawn in terms of the pieces of legislation and guidance documents that underpin the protection of nature and risk assessment protocols in Europe. A conceptual model and all its elements are described as well as the hypothetical exposure routes that may reach three bird species of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*), and Montagu's Harrier (*Circus pygargus*).

Policy Background

Natura 2000 Network

In the Birds Directive [5] it is mentioned that pollution as a result of man's activities may affect birds directly or may destroy their habitats. Whereas for the Habitats Directive [6] the need for Member States developing appropriate management plans with conservation measures is referred. Thus it would be expected that national management plans for Nature 2000 Network sites would foresee in depth all risks posed to the protected species and/or habitats such as chemical pollutants. As will be explained further on with two examples from the Iberian Peninsula this is not the case.

European Risk Assessment Protocols

In 2000 the EU's Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) published an opinion on the scientific basis for proper risk assessment on terrestrial ecosystems [7]. The driving force of this review was the fact that research activities regarding the environmental effects of pollution were dominated by the aquatic compartment and for terrestrial risk assessments the aquatic models had to be adapted. This fact had consequences at the regulatory arena and legislative initiatives considered terrestrial ecosystems of secondary importance or even disregarded it. A clear example lies in the fact that the Water Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy) came out in 2000 whereas the common protection of soils across the EU is yet to be implemented and a proposal for a Soil Framework Directive (COM(2006) 232) was only published in 2006.

Another breakthrough from the CSTEE report was the proposal for a more holistic hazard identification and conceptual model by selecting key route-receptor interactions for each assessment instead of the soil and above soil compartment approach [8]. Exposure scenarios should therefore include the feeding behaviour of species aiming to protect, contaminant concentrations in food as well as transfer of the chemical from soil to food. This way transfer of chemicals into the trophic chain is addressed and hence uptake by animals and plants by bioaccumulation and biomagnification are considered. Although a chemical may pose an acceptable risk for soil dwelling organisms it may represent an unacceptable risk for top predators due to biomagnification through the food chain.

But other important aspects of risk assessment of chemicals on terrestrial ecosystems were also addressed. As indicated by the CSTEE the assessment of effects is mainly aimed on the structure and function of the ecosystem guaranteeing therefore the human uses of the environment (e.g. soil used for agricultural purposes). Thus the protection goal is at population or community level. Nonetheless protected areas with special level of protection for highly endangered species may have to undergo specific risk assessment with the identification of effects at the individual level. Effects requiring an assessment at individual level, such as human health effects, require a different approach that was not considered by the CSTEE review. In fact risk assessment in areas of high ecological concern is still a bit cloudy in EU’s protocols.

Problem Definition and Selection of Scenarios

The foundation of any ecological risk assessment is the clarification of the issue that is going to be evaluated – problem definition – as well as the hazards that will be covered by the evaluation and the respective sources – selection of scenarios [7]. Therefore herewith we will be presenting the environmental values to be protected and describe a model for a targeted, higher tier, risk assessment that includes biological receptors of conservationist concern and the exposure routes for chemicals that are hypothesized to pose risk to the community of birds from a Mediterranean protected area.

Case-study: a Mediterranean SPA

The highest number of plant and animal species in Europe is hosted in the Mediterranean basin, which has been identified by Conservation International as one of the world's 34 biodiversity hotspots [9]. The bimodal weather pattern, that provides the unity to this ecoregion, is dominated by hot, dry summers, and cool, wet winters, with average annual rainfall ranging from less than 100 millimetres in desert territories to more than 4000 millimetres on certain costal massifs. In the western Mediterranean, the Iberian Peninsula, for at least two months each year there is frequently no precipitation al all, and most plants and animals experience a water deficit thus having developed ecophysiological or behavioural adaptations [10].

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. Less than a fifth of the European land can be regarded as not directly managed. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [4]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [11]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems.

An important percentage of the Iberian Natura 2000 Network sites depend upon the continuation of extensive farming practices farmland – Portugal more than 25 %, Spain 18 %, EU-15 18 % –, that are extremely important refuges for several bird species. In December 2006, SPA for wild birds’ sites covered 9.9 % of EU-25 territory but in the Iberian Peninsula this value was of 17 % [12]. Steppic areas are typical examples of HNV farmland from the Mediterranean region with bird assemblages of conservation concern [13]. In the Iberian Peninsula two of the most important sites for the conservation of bird species are, the SPA of Castro Verde (Alentejo, Southern Portugal) [14] and the SPA of the Cereal steppes of Jarama and Henares rivers (North of Madrid, Central Spain) [15]. In both SPAs, extensive agriculture of cereals in rotation with fallow land (normally used as pasture) create a steppic habitat perfect for the conservation of birds such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu’s Harrier (*Circus pygargus*). But the traditional agriculture practices with low intensity non-irrigated cereal farming land represent a marginal economic system with a yield lower than the EU average and specially Atlantic Europe. Hence the main threats to these SPAs are the abandonment of the less fertile agricultural soils with the intensification of agriculture in the remaining land [16; 17].

Ecology of the Receptors

Since the 1970s it has been taking place an overall decline in farmland bird populations across Europe, a declining trend that is not apparent in bird assemblages of other habitats. This long-term trend suggests that the driver factors are specific to this habitat [18] and in fact agriculture intensification may account for the decline of more than 40 % of the bird species [13]. But as evidenced by Donald *et al.* (2006) [18] not all farmland species exhibited patterns of population decline. For the present risk assessment three species of birds were selected: the Great Bustard and the Lesser Kestrel that have a negative population trend of, respectively, -1.1 and -1.39; and the Montagu's Harrier that exhibits a positive mean population trend of 0.64. Thus a typical gregarious omnivorous bird (Great Bustard) and two predators (Lesser Kestrel and Montagu's Harrier) were chosen allowing a different approach in the assessment because the predators, having a larger foraging area obtain food from a relatively larger area with different levels of contamination, and being in the top of the food chain may be exposed to a higher level of contamination due to biomagnification of chemicals.

*Great Bustard (*Otis tarda*)*

The Great Bustard (*Otis tarda*), family *Otididae*, is one of the largest birds of Europe and one of the heaviest flying birds of the world, typical of the steppic habitats and open lands with non-intensive farming [19]. Its populations though widely distributed, from the Iberian Peninsula to eastern Asia, are generally separated and consist of a few tens to several hundred individuals [20]. *O. tarda* is listed in the annex I of the Birds Directive [5] as well as categorized as VULNERABLE in the 2007 IUCN Red List [21].

This species has an accentuated sexual dimorphism with males weighting around 16 kg, which is 2 to 4 times the weigh of females (3-4 kg) [22], and measuring up to 1 m being therefore ca 50 % bigger than females [21]. The Great Bustard is a gregarious bird that lives in flocks with a variable number (4-16) of individuals around the year [22].

Feeding is the most time-consuming activity for the *O. tarda* [23]. They are omnivorous birds that feed mainly upon green plant material, and arthropods and seeds to a lesser extent [19; 20]. In fact green plant material accounts for 71 % in summer and autumn, and 95 % in spring and winter [24]. As for juveniles, fed by rearing females, the diet is based on invertebrates [20].

*Lesser Kestrel (*Falco naumanni*)*

The migratory Lesser Kestrel (*Falco naumanni*), Family *Falconidae*, has a Mediterranean range for breeding, heading south to Africa in winter, particularly to the southern Sahara region. It forages steppic habitats and grasslands with non-intensive cultivation [25]. *F. naumanni* is listed in the annex I of the Birds Directive [5] as well as categorized as VULNERABLE in the 2007 IUCN Red List [25].

This small hawk measures ca 30 cm and seldom exceeds 200 g of weight. Its rusty plumage bears him the right camouflage for the arid habitats where it lives [26; 25]. The Lesser Kestrel is a gregarious raptor that feeds mainly upon insects and preying activity tends occur in the surroundings of the colonies [27].

*Montagu's Harrier (*Circus pygargus*)*

The Montagu's Harrier, Family *Accipitridae*, has a widespread but patchy breeding distribution in Europe, which constitutes over 50% of its global breeding range [28], and it winters sub-tropical Africa and India, and around the Mediterranean Sea [29]. Although it is originally a marsh harrier it colonized the great extent of farmland that covers Europe [30]. *C. pygargus* is listed in the annex I of the Birds Directive [5], and considered of LEAST CONCERN in the 2007 IUCN Red List [28].

Being the smallest within the Harriers, this species measures 43-47 cm [29] and weights around 345 g although males tend to increase in body size as one goes west and south in Europe [30]. It is frequent to observe the reunion of couples in colonies while breeding showing therefore gregarious behaviour [31]. The Montagu's Harrier diet includes small mammals, mainly rodents, and occasionally small birds and large insects [32].

Hazard Identification

Both the management plans from the Portuguese [14] and the Spanish [15] identify the intensification of agriculture as a major threat to the conservation of the birds from the referred SPAs. But due to the present agriculture system chemicals are permitted to use despite the fact that its ecotoxicological evaluation is not foreseen. The exemptions are the prohibition of some pesticides (herbicides and fungicides) in the case of agri-environmental financing under Common Agriculture Policy (CAP) and all chemicals in certain areas as a result of compensation measures following impact assessments of road infrastructures.

This is the case of herbicides applied to the soil before seedling, according to the agronomic application rate, in order to control the competing vegetation. Herbicides are extensively used in agriculture but are negatively correlated with biodiversity [4], namely plants, invertebrates and birds [33].

The existence of livestock grazing in the area creates a less conspicuous pathway for the input of high local concentrations of toxicants into the ecosystem due to the veterinary pharmaceuticals that can be found in dung and urine [34]. The extensive livestock farming is most certainly a vector for this type of contamination. Veterinary medicines are important safeguarding health and welfare of livestock [35] but may have a potential impact in terrestrial ecosystems [36].

Domestic Sewage Sludge is also applied as fertilizer and soil amendment and at the same time as way for the disposal of waste sludge [37]. Previous studies on the use of sewage sludge as fertilizer to soil under Mediterranean climatic conditions revealed an impoverishment of the community structure and decrease in the diversity of microarthropod populations [38; 39]. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [40]. As for the nutrients present in the sludge they are considered to produce negligible risk for the soil compartment [7].

The Conceptual Model

Herewith we present a conceptual model for refining local risk assessments in sites with specific ecological values (figure 2.1). This innovative model is to be used in a high tier risk assessment by taking into account the biotic parameters of bird population from protected areas. The transfer of chemicals is considered to occur mainly through a realistic trophic chain scenario and that is why the different feeding behaviour is considered among different species and even within the same species when having different feeding habits (e.g. adults and juveniles).

According to the management plans of the SPA, the crop production should take into consideration the supply of food for the populations of wild birds. Therefore when birds graze the farmlands or prey upon invertebrates and small mammals that inhabit those fields, are expected to uptake the chemicals that are disposed into soil, through the food chain.

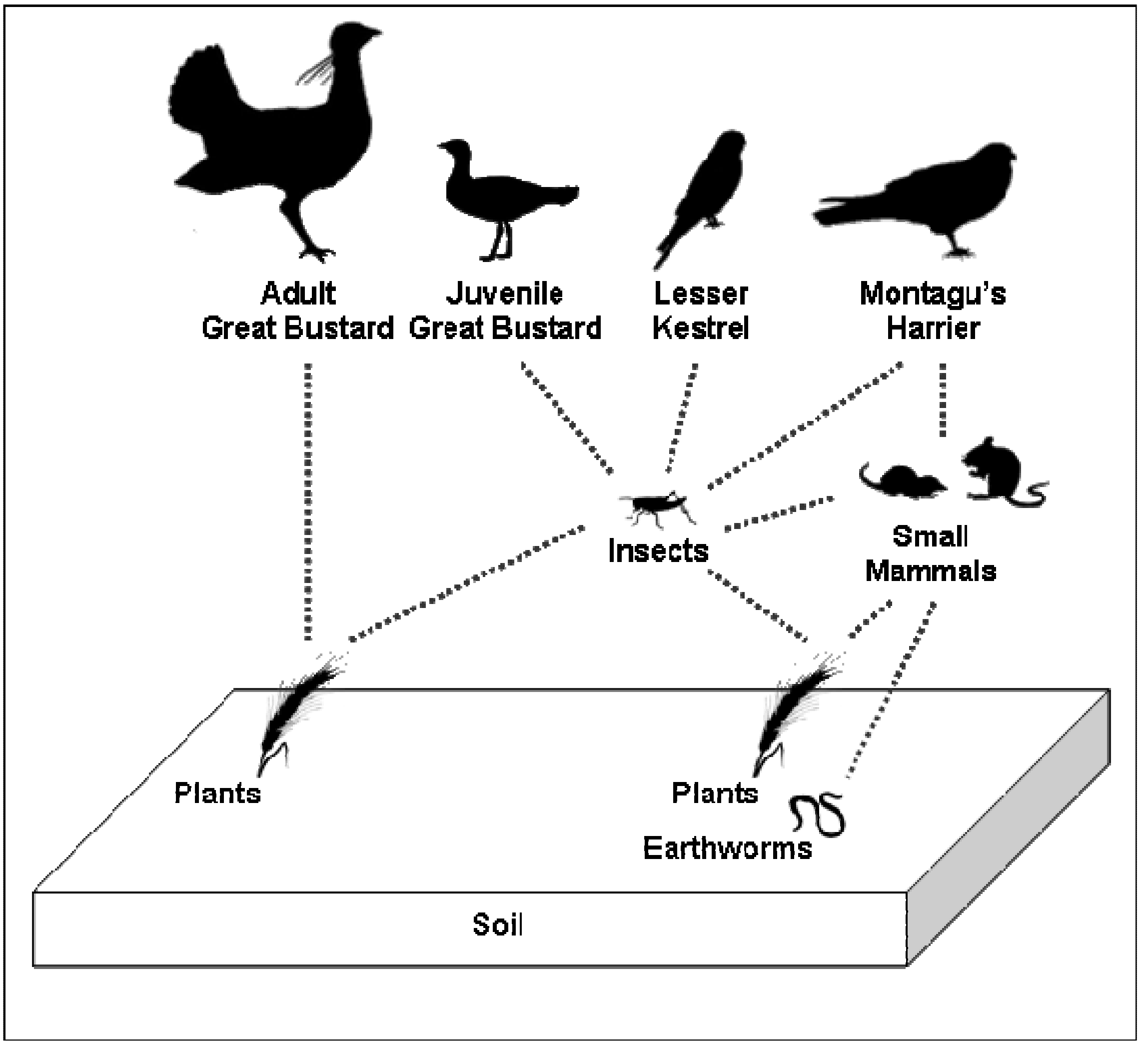

Figure 2.1. Conceptual model for toxicant pathways to the ecological receptors of a food chain from an extensive agriculture habitat.

Before seedling, herbicide for weeds is sprayed in the agriculture fields. In some areas of the farmland, sewage sludge is incorporated into soil. In the areas where grazing takes place, veterinary products are expected to accumulate in soil. Plants and soil-dwelling animals (e.g. earthworms) may bioaccumulate chemicals from soil pore-water [7]. Herbivorous animals – adult Great Bustards, insects and small mammals – may then uptake the chemicals from plants. From insects, the chemicals may pass to the animals that prey them – juvenile Great Bustards (fed by rearing females), Lesser Kestrels, small mammals and Montagu's Harriers. The top predator of the food chain, the Montagu's Harrier, may also uptake the chemicals from preying upon from small mammals that in turn may have been feeding on plants and invertebrates.

Chemicals Assessment

Besides a proper conceptual model, CSTEE [7] also suggests the refinement at exposure and effects assessment steps. Instead of the default values from worst case scenarios real emission data should be used and presented in the form of probability functions for the predicted environmental concentrations. For studying the effects a combined approach of bioaccumulation tests in terrestrial microcosms [41; 42] may be performed in order to attain information on the interactions between different trophic levels, as well as on the transfer of chemicals through the trophic chain.

Risk assessment in protected areas should be used for regulatory purposes in order to safeguard its ecological values as defined by the directives that underpin the Nature 2000 Network. Hence the assessment of chemicals in those sites must be developed in accordance to the generic protocols described in the EU pieces of legislation and guidance documents. In the Annex VI of the Directive 91/414/EEC the detailed evaluation and decision making criteria for plant protection products (e.g. herbicides) is described [43]. Additional technical guidance is presented in the Guidance Documents on Terrestrial Ecotoxicology [44] and Birds and Mammals [45] in support of the directive and in the outputs of the recent European Food Safety Authority scientific workshop on the revision of a guidance document on assessment of pesticide risks for birds and mammals [46]. Data for the risk assessment of sewage sludge may be found in the EC Directive 86/278/EEC on the use of sewage sludge in agriculture [47], namely the limit values for heavy metals, but limits for organic compounds are not included. The limit values for organic compounds can only be found in the EC Working Document on Sludge [48] on the revision of the Directive 86/278/EEC. Nonetheless methodologies for the risk assessment of metals and organic compounds are described in the EC Technical Guidance Document of 2003 [49]. Similarly, veterinary medicines are covered by Directive 2004/28/EC [50] and Regulation (EC) No 726/2004 [51]. In 2007 the European Medicines Agency launched a guideline [52] on the assessment of veterinary medicines in support of two other guidance documents on the environmental risk assessment of veterinary pharmaceuticals adopted following the international harmonisation process through the Veterinary International Conference on Harmonisation [53; 54].

Probabilistic Risk Assessment

In a general way risk assessment methodology is based on the systematic and tiered comparison of the exposure (predicted environmental concentration – PEC) against the effects (predicted no effect concentration – PNEC) with the application of safety factors to account for uncertainty [2]. But as Calow [55] has pointed out already 15 years ago, when looking at the challenges for ecotoxicology in Europe "this is not quite risk assessment in the sense of explicitly characterizing the probability of populations or communities becoming impaired to defined extents". A way to handle this bias is to include ecological considerations in risk assessment [56] or by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [57]. The last is called the probabilistic risk assessment approach where instead of point estimates a distribution for exposure (exposure/environmental concentration distribution) and/or effects (species sensitivity distribution), and concomitant risks may be obtained [58]. Probabilistic methodologies have been considered valid and scientifically sound or have been putted forward by many international bodies involved in the field of risk assessment, e.g., EC [2], SETAC [59], ECETOC [60], OECD [61] or USEPA [62]. And in an European Workshop on Probabilistic Risk Assessment for Pesticides [63], the main advantages of this approach were highlighted to aquatic organisms, and terrestrial plants, vertebrates and invertebrates: helps to quantify variability and uncertainty, can produce outputs with more ecological meaning (e.g. probability and magnitude of effects), makes better use of available data, identifies most significant factors contributing to risk, can provide an alternative to field testing or helps focus on key uncertainties for further study in the field, and promotes better science by considering multiple possibilities. Moreover when considering the probabilistic methods instead of the regular deterministic approach, risk assessment is more transparent, with the sources of uncertainty identified, allowing therefore a clearer communication of risk [7]. Thus another refinement to the assessment of the present conceptual model may be the utilization of the probabilistic approach.

Final Remarks

The nature conservation instruments across Europe that settle the Nature 2000 Network and respective management plans in each Member State completely disregard ecotoxicological tools for setting the impact of pollution on biota. On the other hand, risk assessment methodologies from EU legislation and respective guidance documents are generic protocols that may not protect the ecological values from areas of conservationist concern. For instance the adaptation of the methodologies and basic principles of the EC Technical Guidance Document on Risk Assessment [49] to the biology and ecology of the species that represent the ecological values will allow a suitable assessment of chemicals in designated areas. Furthermore has shown by Faber [64], with a site-specific approach for risk assessment, by differentiating the level of protection for the chosen effect criteria depending on land use, a greater relevance of results will be achieved.

Therefore the development of innovative conceptual models with realistic exposure scenarios may be used to assess the risk of chemicals in protected areas such as SPAs for wild birds. Moreover there is a need for refinement of targeted assessments by taking into consideration the specificities of the Mediterranean ecoregion, and addressing real emission data with the occurrence of biomagnification through the foodchain where protected bird species are ecological receptors. Hence probabilistic methodologies have been putted forwarded as a way to perform transparent risk assessment, clearer risk communication, use of all available data, and to identify the main sources of uncertainty [7].

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CHAPTER 3

Chapter 3. Assessing the risk of cadmium to birds from sludge applications in an European Mediterranean protected area

Abstract

While supplying some essential plant nutrients and enhancing soil organic matter, sewage sludge may be responsible for the input of heavy metals to the terrestrial compartment. Using Cadmium as a model metal, potential avian risks within a bird special protection area (SPA) of the European Natura 2000 Network were assessed. The selected case-study was the SPA of Castro Verde, Southern Portugal (Alentejo), the most important Portuguese area for the conservation of steppic bird species. Terrestrial microcosms were used for studying the bioaccumulation along the food chain of bird species of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). A deterministic scenario and four probabilistic scenarios with increasing Cd concentrations were assessed. Main differences among risks posed to birds are due to diet and concomitant pathways of Cd through the food web. The most critical food chain contribution is that related to the exposure of the top predator Montagu's Harrier, followed by those associated to the exposure of the juvenile Great Bustard, the Lesser Kestrel and the adult Great Bustard, respectively.

Keywords: probabilistic risk assessment, protected area, agriculture, sewage sludge, cadmium, *Otis tarda*, *Falco naumanni*, *Circus pygargus*.

Introduction

The special protection area (SPA) of Castro Verde in southern Portugal, Alentejo, is classified under EU's Nature 2000 Network for the conservation of endangered birds in accordance to the Council Directive 79/409/EEC (birds' directive). According to the Portuguese Institute for Nature Conservation [1] this SPA is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu's Harrier (*Circus pygargus*). The SPA of Castro Verde includes six municipalities of which the municipality of Castro Verde has the higher percentage of land, 55 %, out of a total area of 79007.17 ha. In the last forty years average annual temperature from this region was approximately 22 ºC and annual rainfall 500-600 mm. For the same time span, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [2]. The main landscape is characterized by a mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [3; 4].

In recent years in Castro Verde, wastewater sludge was used as fertilizer in a program aiming to prevent desertification and soil erosion [5] but these products were not assessed for the risks to the ecosystem in spite of the performed chemical analysis. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [6]. The presence of metals in sludge is a clear concern and is regulated by the Sewage Sludge Directive 86/278/EEC [7]. Cadmium can be used as a model metal. According to the risk assessment report (RAR) for Cadmium (Cd) edited by the European Chemicals Bureau [8] sewage sludge is a minor source of Cd for soils on an average basis; but it is a major source of Cd in soils where sludge is applied.

Terrestrial micro and mesocosms have been pointed out as important tools for higher tier risk assessments by gaining insight on the interactions between different trophic levels, as well as on the transfer of chemicals through the trophic chain [9]. Some ecosystem surrogate methodologies have been developed and presented. Terrestrial Model Ecosystems (TME) consist of enclosed intact soil-cores containing biota (plants, animals and microbes) from selected field sites [10] whereas in the Multi-Species Soil Systems (MS 3) columns of natural sieved and homogenised soil are used being the organisms (plants and invertebrates) deployed afterwards [11]. Further refinement on risk assessment may be performed using probabilistic methodologies by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [12]. Thus instead of point estimates a distribution for exposure (exposure/environmental concentration distribution) and/or effects (species sensitivity distribution), and concomitant risks may be obtained [13].

This paper evaluates the hypothesis that Cd present in sewage sludge may undergo bioconcentration through the food chain and pose risk to some bird species of conservationist concern from the SPA of Castro Verde. For the purpose a conceptual model previously presented [CHAPTER 2] was used to assess, with increasing refinement, the risks of Cd metal. Terrestrial microcosms were used for studying the uptake from the different organisms of the food chain reaching the considered ecological receptors: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*).

Methodology

Soil Characterization

Agriculture soil collected from the top 10 cm layer in a field from the SPA of Castro Verde was sieved *in situ* with a 4 mm mesh. A site at the "Herdade de Vale Gonçalinho" (N 43º 14'21 6'', W 8º 30'35 3'') was chosen that had not received manure, sewage sludge or pesticide applications during the last decade. When brought to the laboratory, the soil was left at dark and aerated conditions for one week. The soil was characterized for basic pedological descriptors such as coarse sand (23.3 %), fine sand (37.0 %), silt (24.9 %), and clay (14.8 %); and physical-chemical properties: pH (6,1), residual humidity (4 %), density (1.21), maximum water holding capacity (27.55 %), NH_4^+ content (1 ppm), oxidizable C (1.74 %), total organic matter (3 %), extractable P (60 ppm) and extractable K (98 ppm), in accordance to the methodology proposed by the British Society of Soil Science [14].

Experimental Design

Test species

Three species of birds included in annex I of the Birds Directive [15] were chosen as model ecological receptors: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). While the first two have had a decreasing trend in Europe the last has shown a slight increase in recent years [16]. A conceptual model for the assessment of risks to these birds was presented elsewhere [Chapter 2]. In short the model is based in the diet of each species and the respective food chain (figure 3.1). Adult Great Bustards feed on plants and juveniles eat the insects rearing females provide them. The raptors, Lesser Kestrel and Montagu's Harrier, prey upon insects, and insects and small mammals (herbivorous and insectivorous), respectively. Hence *C. pygargus* may be exposed to higher levels of contamination due to bioaccumulation of chemicals through the food chain. Moreover, birds of prey have larger foraging areas and may obtain food from places with different levels of contamination.

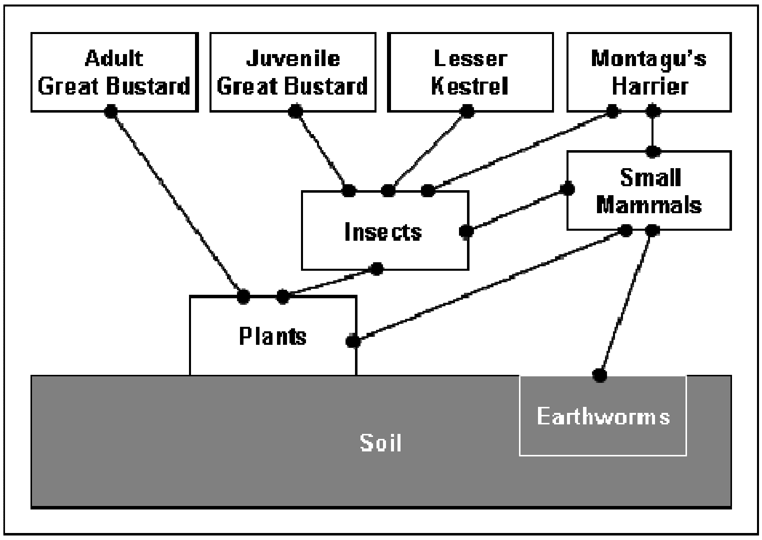

Figure 3.1. Food chain of the selected ecological receptors (protected bird species) from the SPA of Castro Verde.

Laboratory tests were performed with plants and invertebrates, as key elements of the food web, in order to determine the bioaccumulation factors (BAF) for each taxonomic group. Plant species existing in the SPA of Castro Verde were chosen in accordance to *O. tarda* feeding preferences: common wheat (*Triticum aestivum*), chickpea (*Cicer* *arietinum*), and radish (*Raphanus* sp.) [17-19]. But these plant species are also important in the local agricultural scheme since *T. aestivum* is sown in the first year of the rotation system as the primary cereal (in the second year the secondary cereal is sown, oat *Avena sativa*, following two or three years of fallow depending on soil fertility) [1]. Cereals are usually sown in September-November and harvested in June-July [20] and leguminous crops (*C. arietinum*) are sown in smaller amounts in summer. A typical Mediterranean weed *Raphanus raphanistrum* was also intended to be tested but since no commercial seeds of wild radish were available cultivated radish (*Raphanus sativus*) was used instead. Earthworms *Eisenia andrei*, cultured in our lab, were tested as key elements involved in secondary poisoning of organisms that feed upon them [8], and due to the role they play in water infiltration and storage and soil aeration [21] thus contributing for the mobilization of metals [22]. Insects from the order Orthoptera are important food items for juvenile *O. tarda* [18] but also to *F. naumanni* [23] and to a less extent to *C. pygargus* [24]. The locust *Schistocerca gregaria*, late first or early second instars, were acquired from Blades Biological Ltd (http://www.blades-bio.co.uk/) and left to acclimate to laboratory testing conditions during one week while fed *ad libitum* with dry bran and fresh grass.

Terrestrial microcosms

For the experiments with terrestrial microcosms, equipment from the terrestrial model ecosystem (TME), field validated and ring-tested in an EU project [10] ("The use of TME to assess environmental risks in ecosystems", Project No: ENV4-CT97-0470), was used. Soil is contained in 40-cm long high-density polyethylene tubes (17.5 cm diameter) with a plate of the same material at the bottom, and a thin inert gauze to fit between the drilled holes of the bottom plate. The TME tubes are placed into moveable carts; in each cart up to 18 cylinders may be placed and the temperature inside may be controlled with a cooling unit in order to adjust soil temperature. Rain-heads made out of plexiglass (16.5 high and 14 cm diameter, with 12 evenly spaced holes where micro-pipettes are inserted) may be positioned above TME tubes for watering the soil and simulate rainfall.

Originally this equipment is used with intact soil cores, extracted from field without disturbing soil organisms and layers, being the encased soil-cores defined as TME [25]. But instead, for the present experimental work, soil was sieved as proposed by Fernandez *et al.* [11] for the Multi-Species Soil Systems (MS·3). Thus according to the SETAC workshop on "Semi-field methods for the environmental risk assessment of pesticides in soil" [26] sieving draws the line between microcosms and mesocoms and therefore in the methodology presently addressed the term TME cannot be used. This methodological alternative offers a compromise between cost, realism and reproducibility [22]. While overcoming the high variability of mesocosms [27] it reasonably surrogates agricultural (arable) soils where structure and biota are modified [11; 22].

Experimental set-up

Laboratory conditions were adjusted to simulate environmental conditions from spring and autumn in order to address climatic circumstances when, respectively, chickpea and common wheat are sown: photoperiod with light/dark cycles of 10/14 h (8000 lux light intensity, provided by Philips SON-T Agro high pressure sodium lamps); soil temperature 12 ± 2 ºC; laboratory temperature 20 ± 2 ºC; air moisture inferior to 40 %; and rainfall simulation with 150 ml, two times per week, in each TME (total 1.2 l simulating monthly ca 55 mm rainfall). Under Mediterranean conditions a great resemblance exists in weather patterns of spring and autumn.

The experiment was carried out for 28 days with the following scheme:

- At day -1, soil columns were prepared with 10 kg of sieved soil saturated with a volume of 1.3 l of distilled water to get ca 70% of the soil water holding capacity. Before soil was placed into the treatment TMEs it was contaminated by dissolving Cd (cadmium chloride, CAS No: 25155-30-0) in distilled water that was then mixed with soil.
- Seeds (15 chickpea, 20 common wheat and 20 radish) were sown as proposed by OECD guidelines [28; 29] and 10 pre-weighted adult earthworms (with clitellum) were deployed as described in an international ring test for Cd bioaccumulation [30], per column at day 0. Six columns were allocated to the control and other six were allocated as treatment columns per cart. Four carts were used giving a total of 24 control and 24 treatment replicates.
- At day 14 three locusts were deployed per column; in one column per control and treatment in each cart, no locusts were deployed in order to evaluate plant growth and metal concentration at the end of the experiment.

- In the end of the experiment, day 28, aerial part of the plants was cut off and weighed; locusts were collected for survival assessment and the ones that survived were weighted and analysed for the presence of Cd. The soil columns were removed from the cylinder and homogenised in order to take samples that were analyzed for humidity and Cd. Earthworms were collected for survival assessment and the ones that survived were weighted and analysed for the presence of Cd.

Cd Analysis

For the analysis of Cd, soil, plants, invertebrates and leachate samples were digested with HNO_3 suprapur by means of microwave-assisted extraction following the US EPA evaluation methods [31-33]. Cd concentrations were then determined with Atomic Absorption Spectometry (Graphite Furnace AAS; Perkin Elmer Model Analyst 800).

Risk Assessment – scenarios and probabilistic assumptions

The chemical analysis of the wastewater sludge used in Castro Verde indicated a Cd concentration of 3.3 mg $Kg^{-1}{}_{Sludge\ dw}$ [5]. Firstly a worst case scenario (scenario 1) was assessed by considering a concentration of Cd in soil of the same order of magnitude from sludge; thus a concentration of 5 mg $Kg^{-1}{}_{Soil\ dw}$ (4 mg $Kg^{-1}{}_{Soil\ ww}$) was tested in microcosms allowing to determine environmental concentrations in each compartment and the bioaccumulation factors for plants and invertebrates. Afterwards the refinement of exposure assessment (Predicted Environmental Concentration, PEC) with the probabilistic approach was developed by:

- Using a realistic scenario for the exposure of Cd (scenario 2). According to the managers of the project of desertification prevention in Castro Verde [5], a range of 32-40 m^3 ha^{-1} of sewage sludge was amended to soil which meant the amendment of 5-6 T ha^{-1} at depth of 30-75 cm, in sites with total area of 2 ha. The value of Cd estimated to be deployed per ha was of ca 4 g, which represents a concentration of 0.5 µg $Kg^{-1}{}_{Soil\ ww}$.
- Considering the maximum level of Cd permitted by the Sewage Sludge Directive 86/278/EEC [7] (and national legislation, n.º 118/2006 [34]) to be added to

agricultural land (scenario 3), which is of 150 g ha^{-1} per year, that corresponds to a concentration of 19.6 µg $Kg^{-1}_{Soil\ ww}$.

- Modelling the realistic scenario and adding the concentration of Cd from the PEC for a generic Regional environment (PECregional) calculated in the RAR [8] based on the mass balance of Cd including detailed Cd immision onto soil from atmospheric deposition (scenario 4). Thus adding 48 µg Kg^{-1}_{Soil} to the realistic scenario means a final concentration of 48.5 µg $Kg^{-1}_{Soil\ ww}$.
- Covering temporal variation (scenario 5) from the experimentally obtained data by including increasing PEC values over time in plants and invertebrates due to accumulation. As an assumption Cd was considered to be continuously uptaken over time.

BAF values for plants and invertebrates were obtained from a concentration of Cd in soil of the worst case scenario, i.e. 4 mg $Kg^{-1}_{Soil\ ww}$. For the refinement scenarios BAFs were assumed to be the same as in the higher soil Cd concentration.

The formulas used for the calculation of exposure assessment were adapted from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [35]. BAF for plants and invertebrates were obtained: from the organism/soil Cd concentration ratio for plants and earthworms, and from locusts/plants ratio in the case of *S. gregaria* observed in our experimental study. Considering the limited exposure duration, the highest values were employed as representative. For small mammals, the accumulation of Cd increases with time, thus whole body BAF values were calculated with the following formula, $BAF = \alpha * F * ((1 - k_2) * Lifespan)$, where α is the fraction of ingested dose that is absorbed, F is the food intake rate per body weight (calculations are presented in the annex 3.1, table A.3.I), and k_2 is the rate constant for depuration; α and k_2 were obtained from the values presented for mammals in the RAR for Cd, being the k_2 corrected by the *Lifespan*, the maximum period of time that mammals can accumulate Cd [8]. PEC calculations for the ecological receptors are given by the formula: $PEC_{Bird} = (FIR/bw) * C * PD$, where C is the concentration of Cd in fresh diet and PD is the fraction of food type in diet [36].

For the assessment of effects – Predicted No Effect Concentrations (PNEC's) – of the target birds, the value suggested from the Cd RAR [8] was used following the $PNEC_{oral}$ for birds due to secondary poisoning, i.e. 0.16 mg kg^{-1}_{ww}. When characterizing the risk, a safety of factor of 10 was applied for covering the variability within bird species following the principles of the Technical Guidance Document on Risk Assessment [37] and considering that as the assessment is based on doses instead concentrations, the additional factor of 3 for covering lab to field differences in the food energetic content is not required here.

Probabilistic assessment was developed with Crystal Ball software [38] for Monte Carlo Analysis with 10000 trials. In scenarios 2 to 4, BAF values and respective standard deviations for plants and invertebrates calculated from concentrations obtained in the microcosms experiments were set as assumptions with normal distribution (figure 3.2) (annex 3.1, figure A.3.1). Also in scenarios 2 to 4, BAF for small mammals are dependent of the age of its populations; hence a triangular distribution was defined from weaning to maximum lifespan with the likeliest age of two thirds of the lifespan (figure 3.2) (annex 3.1, figure A.3.2) (age values obtained from Blanco *et al.* [39]). In the fifth scenario the temporal scale was considered with consequent increase in Cd uptake; this was modelled by considering a linear distribution assumption in plants and invertebrates, where the lowest value is the baseline concentration (given by control concentrations) and the highest value corresponds to concentration measured in treatment in the last day of the experiment (figure 3.2) (annex 3.1, figure A.3.3).

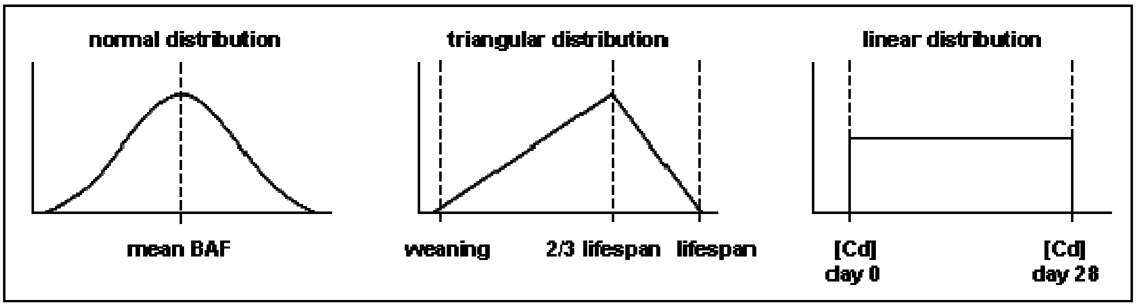

Figure 3.2. Probabilistic assumptions defined for the Monte Carlo Analysis of the scenarios.

Statistics

The comparisons between microcosms in terms of biological parameters, were analysed using one-way ANOVA with the SigmaStat statistical package [40]. Statistical analysis was carried out with a significance level of $p < 0.05$.

Results

Bioaccumulation

In order to determine Cd concentration a minimum quantity of material was needed – 2 g of soil, 150 mg of earthworm, 200 g of plant and 150 g of locust – what limited the usage of microsoms from carts in separate. Since the quantity of soil and *E. andrei* biomass was enough, Cd concentration was analysed in all carts. Plants and *S. gregaria* were only analysed for Cd in two carts.

Cadmium concentration in control soil (table 3.I) is quite low (0.057 mg kg^{-1}_{dw}) when compared with baseline concentrations for agricultural soils from another Mediterranean region (0.7 mg kg^{-1}_{dw}) [41], but exactly matches the background concentrations presented in the Cd RAR at the regional level (0.048 mg kg^{-1}_{ww}) [8]. The highest concentrations of Cd in control organisms are present in soil dwelling *E. andrei* where metal was analysed after guts were voided.

Table 3.I. Cd concentrations (wet weight) in soil and in the organisms from control and treatment (± standard deviation) microcosms, and bioaccumulation factors (wet weight) for plants and invertebrates.

Cd Concentration (mg kg^{-1}_{ww}) in Control Microcosms					
		Plants			
Soil	***E. andrei***	***C. arietinum***	***T. aestivum***	***R. sativus***	***S. gregaria***
0.048	0.458	0.003	0.039	0.041	0.099

Cd Concentration ± SD (mg kg^{-1}_{ww}) in Treatment Microcosms					
Soil	***E. andrei***				
4.18 ± 0.36	17.16 ± 2.88				
		Plants			
Soil		***C. arietinum***	***T. aestivum***	***R. sativus***	
4.21 ± 0.30		0.71 ± 0.38	5.66 ± 2.01	4.14 ± 2.36	
		Plants			
		C. arietinum	***T. aestivum***	***R. sativus***	***S. gregaria***
		0.64 ± 0.32	5.08 ± 1.42	4.18 ± 1.64	17.21 ± 5.36

BAF ± SD (kg_{ww} kg^{-1}_{ww})					
		Plants			
	E. andrei	***C. arietinum***	***T. aestivum***	***R. sativus***	***S. gregaria****
	4.13 ± 0.77	0.17 ± 0.10	1.36 ± 0.52	0.98 ± 0.50	1.67 ± 0.08

*** BAF for locusts was calculated taking into consideration its feeding preferences, 63 % *Cicer*, 14 % *Triticum* and 23 % *Raphanus*.**

BAF values are higher in *E. andrei* (table 3.I) than in the other tested invertebrate *S. gregaria*, which is in accordance with the trend of bioaccumulation factors reviewed in the RAR for earthworms and arthropods [8]; Cd is also concentrated (BAF > 1) in wheat. In previous studies on the bioaccumulation of Cd through the food chain [42; 43] its bioconcentration from soil to *T. aestivum* and to wheat phloem-feeding feeding aphids was also demonstrated. In some microcosms no locusts were deployed, giving us and average plant biomass growth. The difference between the average growths in microcosms with locusts allowed the inference of the feeding preferences of *S. gregaria* that had to be taken into account for the BAF calculation because the different tested plant species bioaccumulate different concentrations of Cd.

Deterministic BAF values for small mammals are as follows: herbivorous, weaning 0.2 kg kg^{-1}, two thirds of lifespan 2.1 kg kg^{-1} and lifespan 3.1 kg kg^{-1}; insectivorous, weaning 0.6 kg kg^{-1}, two thirds of lifespan 6.6 kg kg^{-1} and lifespan 9.9 kg kg^{-1}.

The EU RAR [8] and other ecotoxicological studies on contaminated land (e.g. [44-48]) offer a review of the accumulation pattern of Cd and basically conclude that in general, the concentration in the exposed organisms increases with the exposure time and experimental conditions and age in field studies, suggesting the steady state is not, at least rapidly achieved. In addition, the bioaccumulation factors tend to decrease with the increase in the soil cadmium concentration. As a consequence, the selection of the BAFs represents a critical element for the risk assessment. Two complementary approaches have been used in this study. First, the maximum BAF obtained in the experimental study for each species; second, an alternative to the BAF approach, using actual measured concentrations including the temporal variability. Both approaches were selected after considering the exposure route assessed in this study, and are expected to offer worst-case potential exposure conditions. The experimental BAFs and the increases in concentrations after Cd application were obtained for a relatively high concentration of Cd to the soil, equivalent to that expected in the sludge. This approach represents the worst case situation, unrealistic if an homogeneous distribution is assumed, but potentially realistic during a limited time period for those areas with no or very limited mixture of sludge within the soil, considering also the potential for attraction of soil dwelling animals (particularly relevant as the soil organic matter content is very low) and for significant plant growth (as the sludge offers a huge amount of nutrients). The exposure duration was sufficiently short for avoiding the inverse relationship between concentration and BAF, as demonstrated through the comparison with the BAF observed for the control samples. Hence, the selected approach can be considered appropriate for estimating the maximum potential Cd concentration in biota at the very local level related to sludge applications.

Exposure

PEC results for birds (figures 3.3 e 3.4) are given as point estimates from the deterministic approach – scenario 1 – or exposure distribution of probable occurrences within a defined range of Cd concentration, depending on the scenario, resulting from the Monte Carlo analysis (probabilistic approach) – scenarios 2 to 5. Already in scenario 1 it can be observed that following our hypothesis, at least for Montagu's Harrier, a biomagnification of Cd takes place from soil (4.2 mg kg^{-1}) through the food chain to the top predator (6.36 mg kg^{-1}). In view of the diet described for *C. pygargus* by Corbacho *et al.* [24], the following percentages of food were considered for the PEC calculations: 70 % herbivorous small mammals, 20 % insectivorous small mammals, and 10 % locusts. The refinement of exposure assessment with probabilistic analysis and the increase in Cd soil concentration from scenario 2 to 5 stresses the deterministic results and higher concentrations are present in Montagu's Harrier than in other bird species. As a result of the normal distribution assumptions for BAF in plants and invertebrates pathways for Cd uptake, the frequency of accumulation in birds from scenarios 2 to 4 also follows a normal distribution pattern. In scenario 5 it was assumed that the accumulation of Cd in plants and invertebrates does not always corresponds to the maximum measured value, but can be any concentration within the range measured in the experimental study. The rationale is that the consumer may feed randomly on plants/invertebrates of different ages, and therefore different accumulation levels. This leads to a pseudo-linear distribution of the Cd distribution in birds depending on the soil-plant-locust pathway, i.e. juvenile Great Bustard and Lesser Kestrel. The PEC from adult Great Bustard results only from ingestion of plants and was calculated by considering the different percentage of families present in the diet described by Palacios *et al.* [17] and represented in the present microcosm experiment, Fabaceae (40 %) for *C. arietinum*, Brassicaceae (32 %) for *R. sativus*, and Poaceae (28 %) for *T. aestivum*.

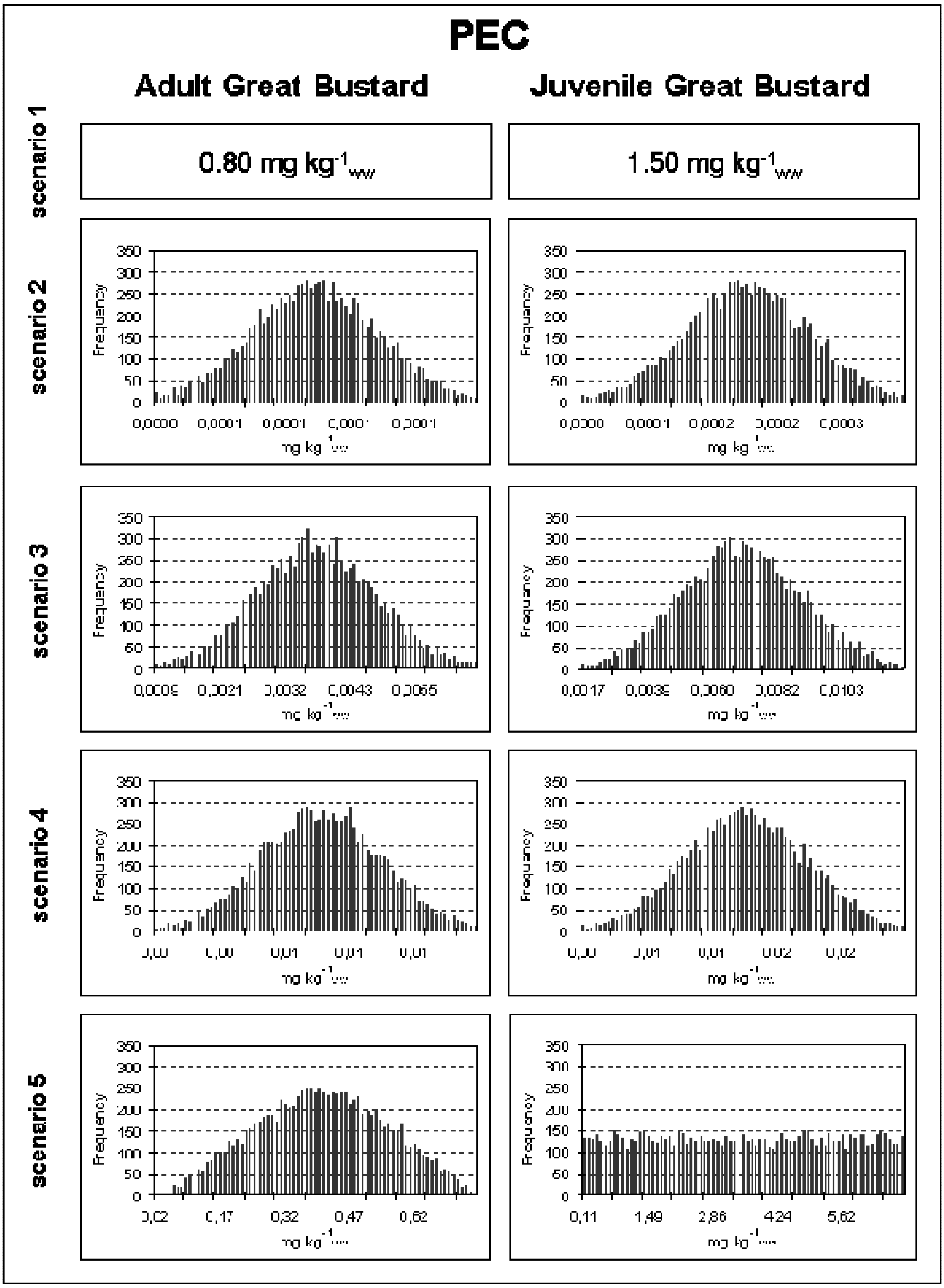

Figure 3.3. PEC (mg kg^{-1} wet weight) for Adult and Juvenile Great Bustards (*O. tarda*) in the five addressed scenarios.

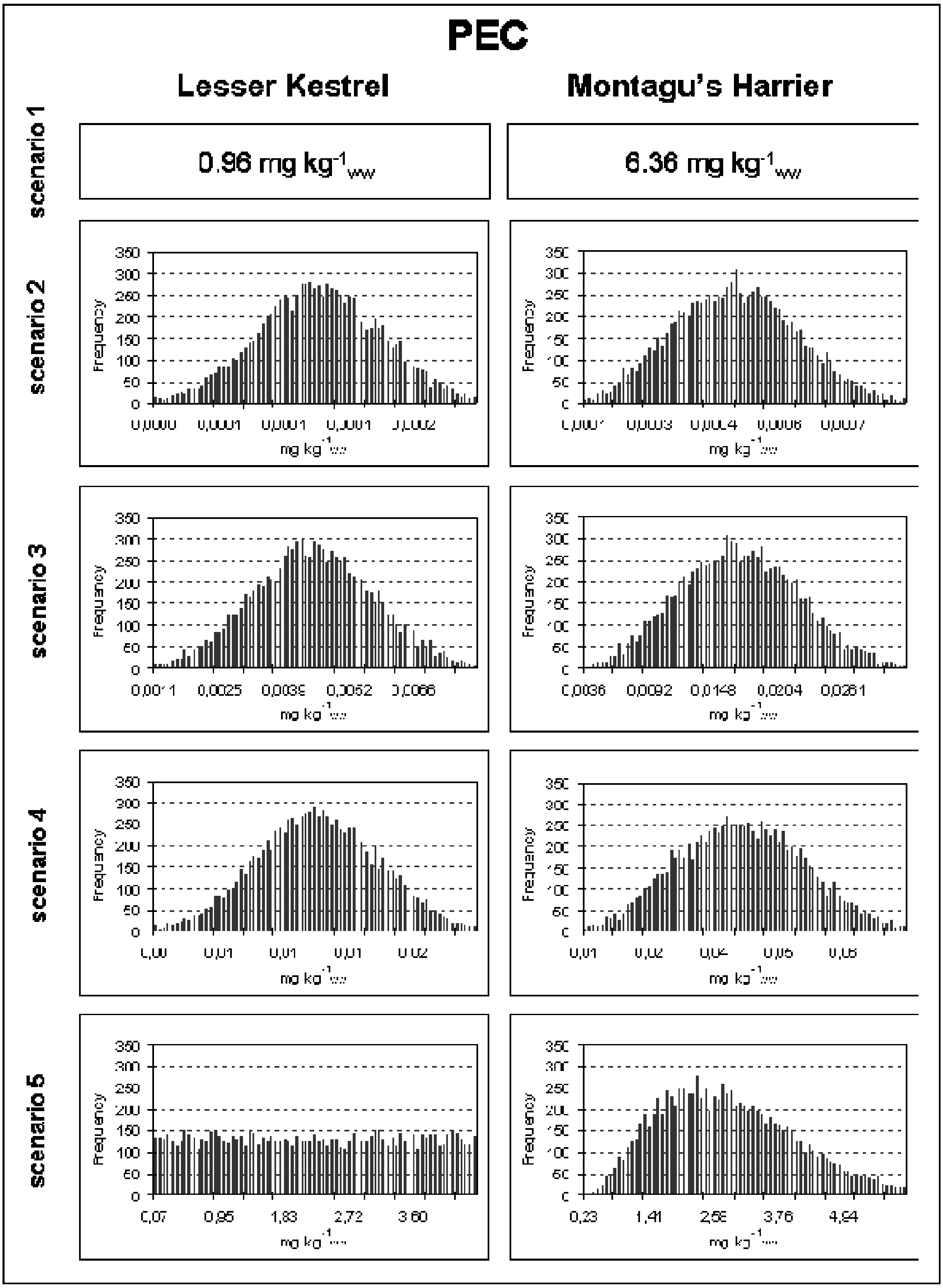

Figure 3.4. PEC (mg kg^{-1} wet weight) for Lesser Kestrel (*F. naumanni*) and Montagu's Harrier (*C. pygargus*) in the five addressed scenarios.

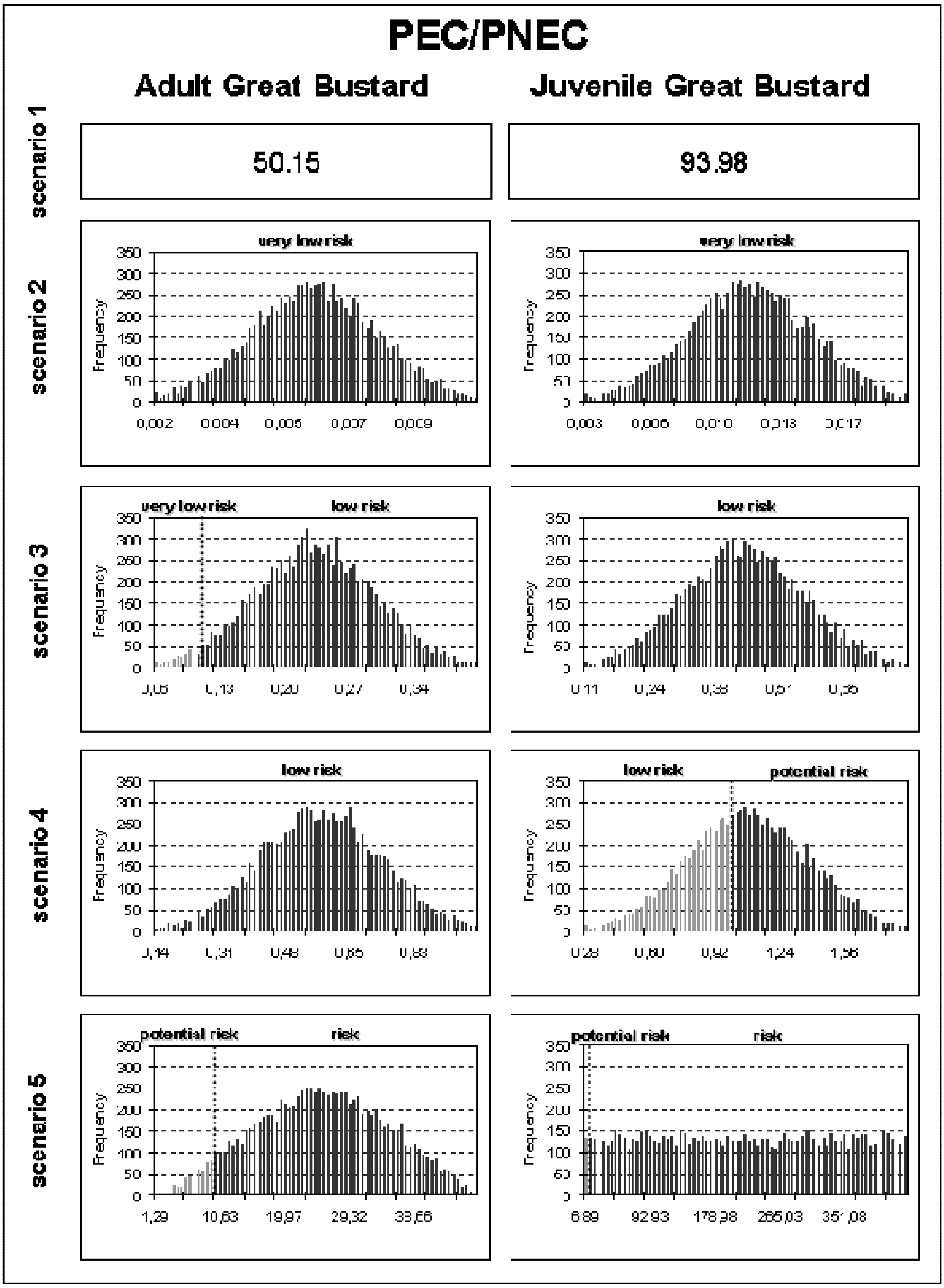

Figure 3.5. Risk characterization (PEC/PNEC) for Adult and Juvenile Great Bustards (*O. tarda*) in the five addressed scenarios.

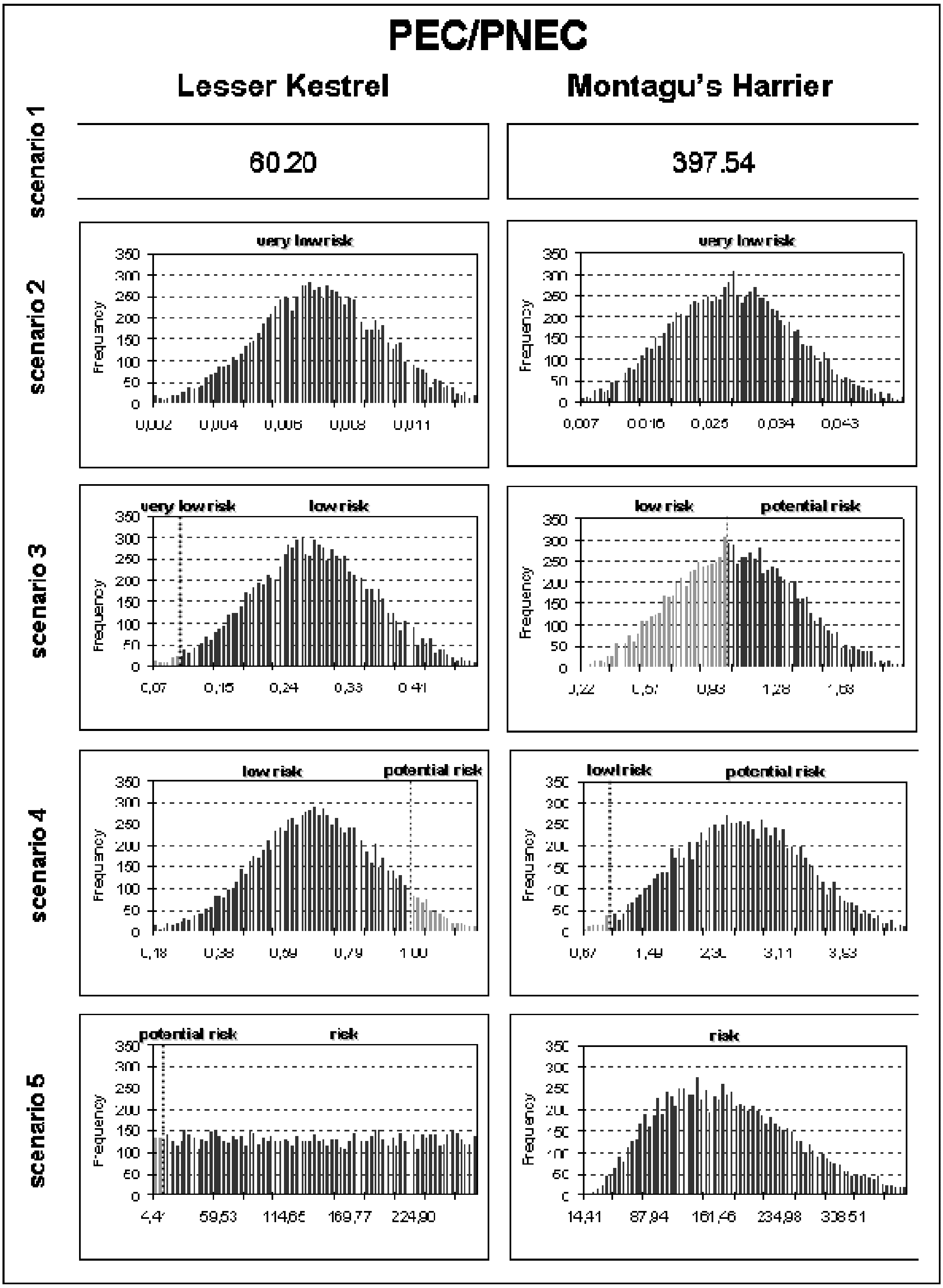

Figure 3.6. Risk characterization (PEC/PNEC) for Lesser Kestrel (*F. naumanni*) and Montagu's Harrier (*C. pygargus*) in the five addressed scenarios.

Risk Characterization

The characterization of risk is based on the comparison of the exposure (PEC) against the effects (PNEC) with the application of safety factors to account for uncertainty [49]. In the case of deterministic approach if the PEC/PNEC ratio is higher than one it is assumed to exist potential risk for the targeted organisms. The first scenario (figures 3.5 e 3.6), considering a worst case where plants are sown directly in the sewage sludge without mixing with agricultural soil, seems to pose risk for birds and particularly to the Montagu's Harrier where the PEC/PNEC ratio is one order of magnitude higher than in the other species.

For the characterization of risk with the probabilistic approach four classes of risk were defined (figures 3.5 e 3.6): (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. Furthermore the frequency distribution allowed the quantification of probability of risk classes with Crystal Ball software [38]. In scenario 2 a very low risk was assessed for all bird species. The third scenario led to an increase in the PEC/PNEC ratio moving the assessment to the class of low risk, remaining a 2.48 % and 1.21 % probability of very low risk in adult *O. tarda* and *F. naumanni*, respectively. But in the case of *C. pygargus* a potential risk of 56.37 % was also obtained. The increase in Cd concentration of soil in scenario 4 resulted in a slight moving forth of the risk classification. While only low risk was assessed for adult Great Bustard, a potential risk due to Cd was found in other birds (6.6 % Lesser Kestrel, 60.59 % juvenile Great Bustard and 98.65 % Montagu's Harrier). In the last assessed scenario the concentrations used were the ones from the microcosms experiments but unlike scenario 1, measured data for PEC in plants and invertebrates was used instead of calculations of exposure with BAF values. Nonetheless for small mammals BAF values were used for modelling PECs. By integrating the time dimension in the assessment as linear distribution of increasing concentrations in *E. andrei*, *C. arietinum*, *T. aestivum*, *R. sativus* and *S. gregaria*, the continuous uptake of Cd was modelled from day 0 (considering baseline concentrations) to the higher concentrations measured at day 28. As in PEC graphs, PEC/PNEC ratio reflect the patterns of the assumptions distributions in plants and invertebrates, especially for birds depending on the soil-plant-locust pathway. In juvenile Great Bustard and Lesser Kestrel there is an uniform distribution of the probability of risk, ranging from potential risk (lower than 10) and, respectively, 98.39 %

of risk reaching values higher than 351.08 and 97.02 % of risk reaching values higher than 224.90.

Discussion

Cadmium is a toxic, nonessential, trace metal that from soil can be rapidly transferred to plants [45; 50], and to invertebrates [44; 46] and small mammals [47; 48] through the food chain. In wild birds, Cd has been measured in raptors [51] and in a grouse species [52], though in low concentrations, showing the possibility of contamination in these animals due to different pathways.

The tested concentrations of Cd seem to be non-toxic for plants and invertebrates, in terms of seedling emergence for *C. arietinum* ($F_{1,30}$ = 0,0826; p = 0.776), *T. aestivum* ($F_{1,30}$ = 1.786; p = 0.191) and *R. sativus* ($F_{1,30}$ = 0,0882; p = 0.769), and in terms of mortality for *E. andrei* ($F_{1,22}$ = 0.0445; p = 0.835) and *S. gregaria* ($F_{1,30}$ = 0; p = 1). In fact, according to the Cd RAR, the Effect Concentration for 50 % (EC_{50}) of an *E. andrei* population (for a 84 day test in a pH 6.3 soil) was of 253 mg kg $^{-1}$, and the median EC_{50} for plants is about 100 mg kg $^{-1}$ [8]. The present worst case deterministic calculations for PEC in earthworms and plants is bellow these thresholds: *C. arietinum*, 0.72 mg kg $^{-1}$; *T. aestivum*, 5.73 mg kg $^{-1}$; *R. sativus*, 4.13 mg kg $^{-1}$; and *E. andrei*, 17.40 mg kg $^{-1}$. A previous study in a Cd contaminated grassland ecosystem revealed concentrations for a grasshopper species (2.4 mg kg $^{-1}$) [44] and an herbivorous (less than 10 mg kg $^{-1}$) and an insectivorous mammal (over 70 mg kg $^{-1}$) [47] at the same order of magnitude than the presently calculated PECs, respectively, 3.68 mg kg $^{-1}$, 3.15 mg kg $^{-1}$ and 104.55 mg kg $^{-1}$. But the increase in the considered trophic levels from adult Great Bustard, to juvenile Great Bustard, to Lesser Kestrel, and to Montagu's Harrier makes clear the contribution of diet and its consequences in risk characterization.

The quantification of probability of risk was possible with the Monte Carlo analysis in scenarios 2 to 5. Sewage sludge amended to agricultural soil in Castro Verde does not seem to pose risk to protected bird species from the SPA assuming a homogeneous distribution of the sludge within the soil, as the Cd concentration in the applied sludge is very low. However, increasing Cd concentrations to the maximum limit permitted by the Sewage Sludge Directive 86/278/EEC [7] (and national legislation, n.º 118/2006 [34]) settles a high probability of potential risk to *C. pygargus*. Furthermore the scenario for Cd concentrations in soil foreseen at the regional level by the RAR [8], that matches the baseline concentration in Castro Verde agriculture soils, also poses potential risk for Lesser Kestrel and particularly to juvenile Great Bustard and Montagu's Harrier.

The ecological parameters and particularly the diet of birds implies differences in the exposure to Cd, as also shown by the probabilistic model developed by Jongbloed *et al.* [53], and hence to the characterization of risks. *C. pygargus*, top predator feeding of all levels of the food chain with organisms with high BAF values, has the most critical food chain for secondary poisoning. Juvenile *O. tarda* and *F. naumanni* share the same food chain but since the first has a higher food intake rate per body weight shows a more critical food chain. Adult *O. tarda* that feeds only on plants has the less critical food chain.

Acknowledgments

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Annex 3.

Table A.3.I. Daily food intake rate per body weight, for wild birds and mammals from the conceptual model. Appendix I, EC (2002)[1].

	Body weight (g)	DEE Equation	DEE (Kj d^{-1})	Food characteristic Food type	Energy (kJ $g^{-1}{}_{dw}$)	Moisture (%)
Adult Male Great Bustard	16000	Other birds	8387,7	Grasses, cereal shoots	18	76,4
Adult Female Great Bustard	3500	Other birds	2593,5	Grasses, cereal shoots	18	76,4
Juvenile Great Bustard	1500	Other birds	1348,0	Arthropods	21,9	70,5
Lesser Kestrel	200	Other birds	284,4	Arthropods	21,9	70,5
Montagu's Harrier	345	Other birds	433,3	90 % bird and mammls 10 % Arthropods	22,5	69,0
Herbivorous small mammals	25	Other eutherians	67,8	Grasses, cereal shoots	18	76,4
Insectivorous small mammals	10	Other eutherians	35,6	Soil invertebrates	19,3	84,6

	Assimil Effic. Food type	%	FIR (fresh material) (g day^{-1})	FIR / bw
Adult Male Great Bustard	Gruiformes Herbage	59	3346,6	0,209
Adult Female Great Bustard	Gruiformes Herbage	59	1034,8	0,296
Juvenile Great Bustard	Gruiformes Animal	34	613,7	0,409
Lesser Kestrel	Falconiformes Animal	84	52,4	0,262
Montagu's Harrier	Accipitriformes Animal	82	75,6	0,219
Herbivorous small mammals	Crops, forbs, mixed vegetation	74	21,6	0,863
Insectivorous small mammals	Insects	88	13,6	1,359

[1] EC (2002). *Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC.* SANCO/4145/2000 - final. Brussels: Europe Commission. Directorate - General Health and Consumer Protection.

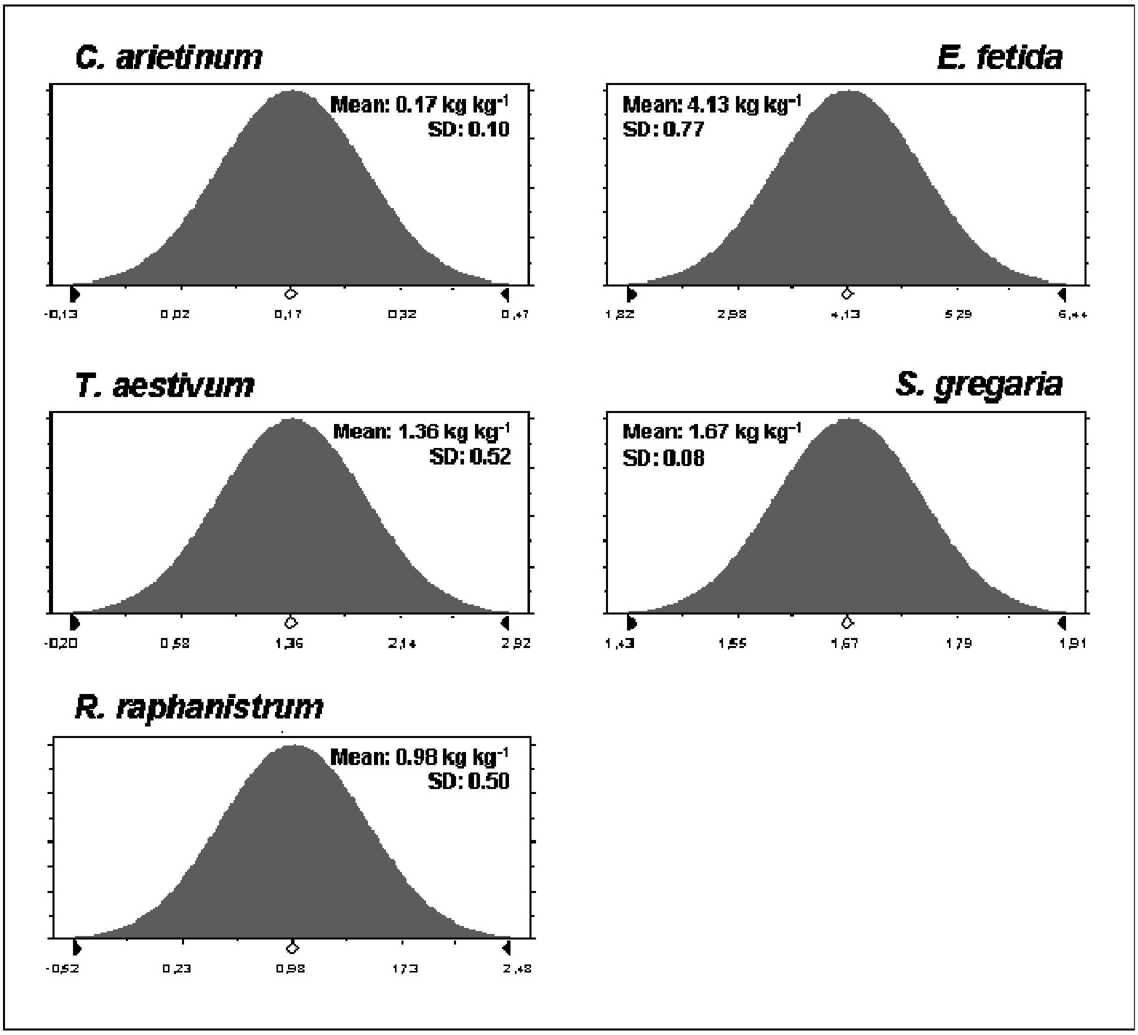


Figure A.3.1. Normal distribution assumptions for the plants and invertebrates BAFs of scenarios 2 to 4.

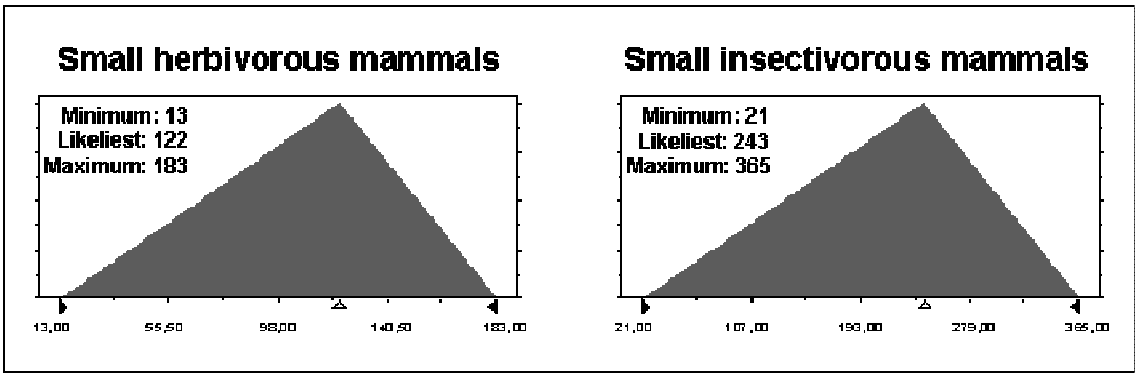


Figure A.3.2. Triangular distribution assumptions for small mammals age populations' (in days) of scenarios 2 to 4. Minimum value is the weaning period, the likeliest age corresponds to two thirds of the lifespan, and the maximum value stands for lifespan.

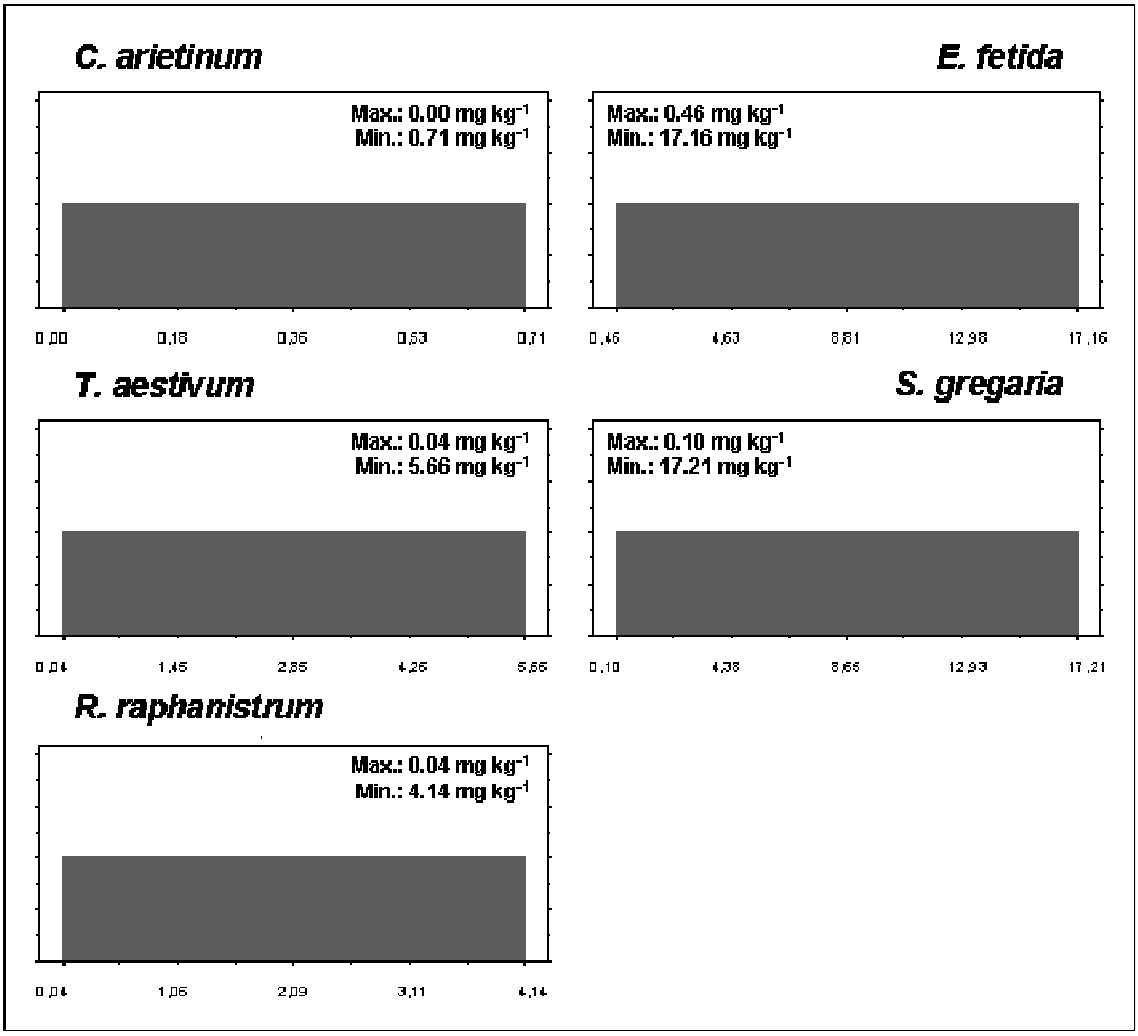
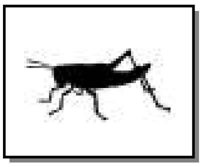


Figure A.3.3. Uniform distribution assumptions for inscreasing Cd concentration in plants and invertebrates, due to uptake over time, of scenario 5.

CHAPTER 4



Chapter 4. Assessing the risk of glyphosate and LAS in an European Mediterranean protected area

Abstract

European species depend to a large extent upon habitats created by man. The traditional forms of agriculture are essential for the conservation of many species, particularly birds. The main landscape of the bird special protection area (SPA) of Castro Verde, integrated in the European Natura 2000 Network, is characterized by extensive farmland of cereals and fallow land. But the traditional scheme of agriculture may still be responsible for the input of toxic chemicals like herbicides and the complex burden of sewage sludge used as soil fertilizer. The herbicide glyphosate (along with its major breakdown product AMPA) and the surfactant LAS, a major organic contaminant present in sewage sludge, were assessed for risks posed to birds of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). Probabilistic approach for risk refinement was used. Real-case scenarios were used for exposure of organic contaminants in soil. While a potential risk of secondary poisoning is expected for birds that have a food chain based in sludge-amended agricultural soils, only a potential risk for juvenile Great Bustards is posed by herbicide usage according to the agricultural application rate.

Keywords: probabilistic risk assessment, protected area, agriculture, herbicide, glyphosate, sewage sludge, LAS, *Otis tarda*, *Falco naumanni*, *Circus pygargus*.

Introduction

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [1]. Following the overall trend, biodiversity in Europe's farmland has declined strongly in the last decades with a special emphasis to bird populations [2]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [3]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems. The intensification of agriculture, and concomitant increase in nutrient and pesticide inputs, has been identified as a major vector to the decrease of biodiversity [4]. The depleting role of herbicides to biodiversity is widely recognized (e.g. [5-9]). Sewage sludge has been used in agriculture as a source of nutrients for fertilizing and soil amendment, but at the same time it may be responsible for the input of toxicants in terrestrial compartment [10]. Recently, the concern on the presence of micropollutants in the sludge has been extended to organic chemicals [11]. A large list of chemicals used in consumer products can be found in the sludge. Detergent components are of special concern in countries such as Denmark [12].

The basis for action of the EU's Biodiversity policy is provided by the Birds and the Habitats Directives, the so-called "nature directives". Across Europe, several sites are classified under the nature directives, Special Protected Areas (SPAs) for wild birds [13] and Sites of Community Interest (SCIs) for habitats and endangered species [14], encompassing the Natura 2000 Network. In December 2006 it already covered more than 20 % of EU-25 territory [15].

The main objective of the present work is to assess the risk of two organic xenobiotics, glyphosate (herbicide) and linear alkylbenzene sulphonate LAS (anionic surfactant, present in sewage sludge), to protected bird species of a Portuguese bird SPA.

A conceptual model based on the food chain of the ecological receptors [CHAPTER 2] will be used to test the hypothesis that the organic pollutants reaching soil are uptaken by plants and then undergo biomagnification and be responsible for secondary poisoning of the target bird species. Probabilistic tools will be used for the refinement of risk assessment.

Methodology

Case study: SPA of Castro Verde

The present risk assessment will consider the SPA of Castro Verde in southern Portugal, Alentejo, as the case study for a farmland site from Natura 2000 Network. Landscape is characterized by a mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [16; 17]. In terms of climatic environment, over the last forty years, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [18]. Extensive agriculture of cereals with fallow and climatic conditions (hot, dry summers, and cool, wet winters) are responsible for bringing forth a steppic habitat characteristic of the most important Portuguese refuge for several bird species of conservationist concern like the Great Bustard (*Otis tarda*) [19], the Lesser Kestrel (*Falco naumanni*) [20], or the Montagu's Harrier (*Circus pygargus*) [21].

Table 4.I. Characterization of agriculture soil from the SPA of Castro Verde

Pedological descriptors			Physical-chemical properties		
Coarse sand	23.43	%	pH	6.1	
Fine sand	36.96	%	Residual humidity	4	%
Silt	24.87	%	Density	1.21	
Clay	14.76	%	Maximum water holding capacity	27.55	%
			NH_4^+ content	1	ppm
			Oxidizable C	1.74	%
			Total organic matter	3	%
			Extractable P	60	ppm
			Extractable K	98	ppm

Soil from the top 10 cm layer, was collected from a site of the "Herdade de Vale Gonçalinho" (N 43º 14'21 6'', W 8º 30'35 3'') that had not received sewage sludge or herbicide applications during the last decade, and was sieved *in situ* with a 4 mm mesh. Basic pedological descriptors and physical-chemical properties were analyzed following the British Society of Soil Science methodological procedures [22] (table 4.I).

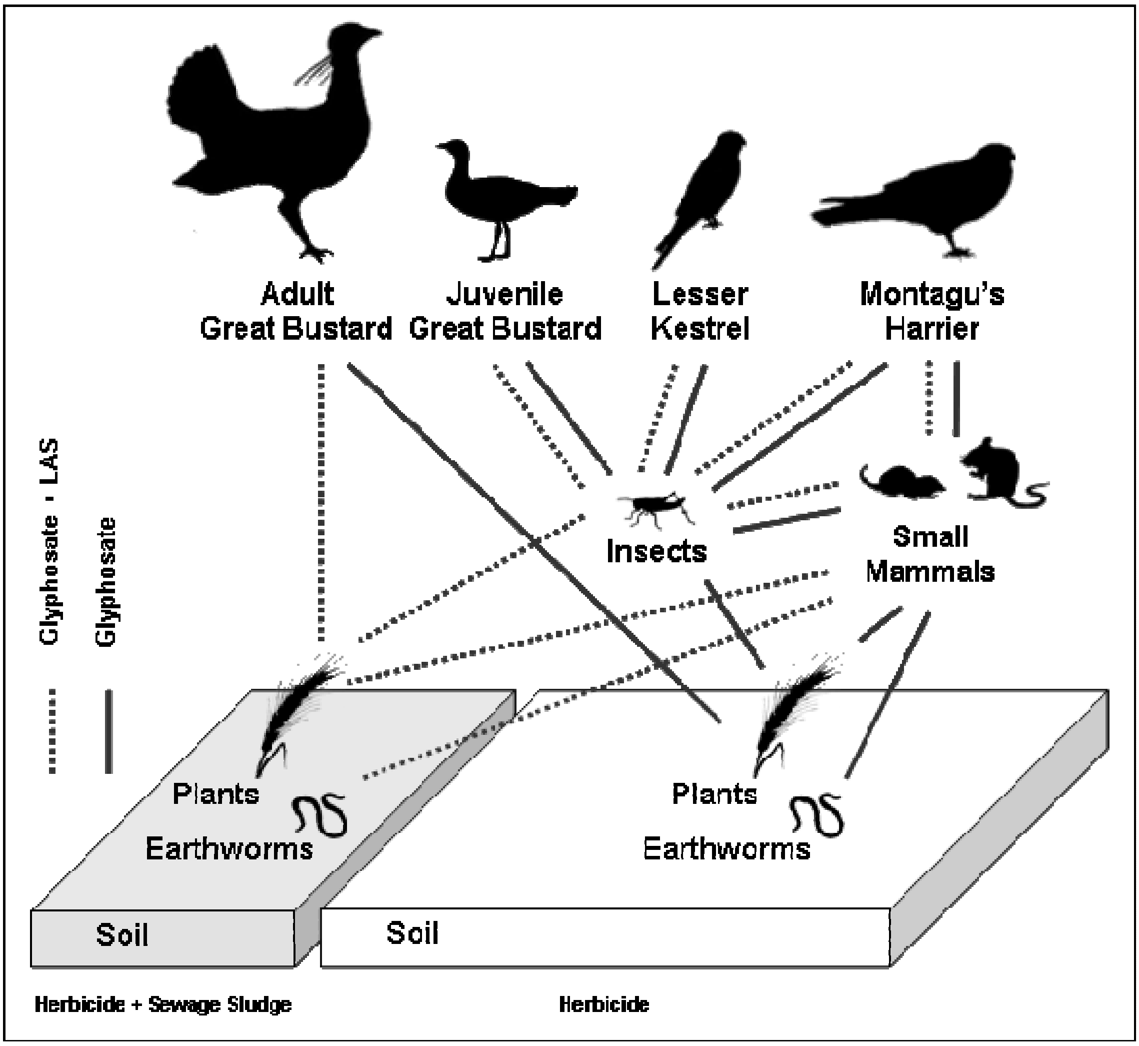


Figure 4.1. Conceptual model for LAS and Glyphosate pathways to the ecological receptors of a food chain from an extensive agriculture habitat (Adapted from CHAPTER 2).

Conceptual Model

The conceptual model (figure 4.1) used to assess the risk of the toxicants from extensive agriculture was described elsewhere [CHAPTER 2]. In short it is based on the effects of secondary poisoning along the trophic chain determined by the diet of the Great Bustard and two raptors, the Lesser Kestrel and the Montagu's Harrier.

In the present experimental work the two organic pollutants assessed with this model were:

(i) Herbicide glyphosate that is marketed as a non-selective, broad-spectrum, post-emergence herbicide [23; 24] and is applied in this farmland area before seedling.

(ii) Linear alkylbenzene sulphonate (LAS) that is the most widely used anionic surfactant in cleaners and detergents and is a major organic contaminant present in sewage sludge [25]; sewage sludge was deployed in limited 2 ha areas from the agriculture fields of the SPA of Castro Verde until 2002 in a program aiming to prevent desertification and soil erosion [26].

Once the organic toxicants reach plants through soil, they may be transferred along the food chain and reach the different considered ecological receptors, depending on the birds diet: from plants, organic pollutants may be bioaccumulated in adult Great Bustards, insects or small herbivorous mammals; small insectivorous mammals may accumulate toxicants from soil dwelling invertebrates e.g. earthworms; from insects, organic pollutants may be uptake by the animals that feed upon them, juvenile Great Bustards, small insectivorous mammals and both birds of prey; from small mammals, organic pollutants may reach the Montagu's Harrier. Hence, assuming that organic toxicants bioaccumulate along the food chain it may be expected higher concentrations in top predators.

Experimental set-up

The role of plants and invertebrates in the transfer of toxicants through the food chain was studied using terrestrial microcosms as surrogates of the ecosystem from the SPA of Castro Verde. For the purpose equipment from terrestrial model ecosystems (TME), field validated and ring-tested in an EU project [27] ("The use of TME to assess environmental risks in ecosystems", Project No: ENV4-CT97-0470), was used. But instead of enclosing intact soil-cores as a mesocosm approach [28] homogenised sieved soil was used instead, as proposed for Multi-Species Soil Systems (MS·3) microcosms [29], thus allowing better reproducibility [29; 30] and overcoming mesocosms' high variability [31]. Plants from the SPA were chosen in accordance to the Great Bustard feeding preferences [32-34]. Two crop species, common wheat (*Triticum aestivum*) and chickpea (*Cicer arietinum*), were studied. According to the traditional rotation scheme, *T. aestivum* is sown as a primary cereal in September-November and harvested in June-July, stage when leguminous crops (*C. arietinum*) are sown in smaller amounts [35]. Wild radish *Raphanus raphanistrum*, is a native weed from the Mediterranean region that has been reported to be resistant to herbicides [36], and its presence has been shown to reduce wheat above ground biomass due to its competitive annual growth habit and high fecundity [37; 38]. But since no commercial seeds of wild radish were available cultivated radish (*Raphanus sativus*) was used instead. Furthermore belonging to the same genus these two species can easily hybridize [37]. Earthworms play an important role in secondary poisoning of small vertebrates [39] and water infiltration and storage and soil aeration [40] thus contributing for the mobilization of toxicants [30]. *Eisenia andrei* cultured were in our lab were tested in the microcosms experiments. Orthoptera were chosen as test insects because they are important food items for juvenile *O. tarda* [33] but also to *F. naumanni* [41] and to a less extent to *C. pygargus* [42]. The locust *Schistocerca gregaria*, late first or early second instars, were acquired from Blades Biological Ltd (http://www.blades-bio.co.uk/) and left to acclimate to laboratory testing conditions during one week while fed *ad libitum* with dry bran and fresh grass.

The day before the beginning of the experiment, 10 kg of soil from the SPA of Castro Verde, saturated with 1.3 l of distilled water, was placed in 40-cm long high-density polyethylene columns (17.5 cm diameter) covered at the bottom with a plate of the same material and a thin inert gauze in between. In the case of treatment columns LAS was dissolved with the distilled water. A commercial formulation of LAS Ufasan 65 (CAS No: 25155-30-0), Unger Fabrikker A.S, with 65 % of active substance by mass and chains containing 11-12 carbon units, was tested. Soil samples were taken for LAS analysis. Columns were then placed in a cooled cart system allowing soil temperature to be at 12 ± 2 ºC. Afterwards Roundup Ultra (Bayer), the most used formulation of glyphosate, was sprayed on the top of soil treatment tubes.

Laboratory conditions were set to simulate climatic conditions of spring and autumn, respectively, when chickpea and common wheat are sown [18]. This meant an average temperature of 20 ± 2 ºC; air moisture inferior to 40 % and light/dark cycles 10/14 h. Lighting of 8000 lux intensity was provided by Philips SON-T Agro high pressure sodium lamps. To simulate rainfall, rain-heads made out of plexiglass (16.5 high and 14 cm diameter, with 12 evenly spaced holes where micro-pipettes are inserted) were placed above soil tubes twice a week with 1.2 l simulating monthly ca 55 mm rainfall per column. Six columns were allocated to the control and other six were allocated as treatment, per cart. Four carts were used giving a total of 24 control and 24 treatment replicates.

At day 0, 10 pre-weighted adult earthworms (with clitellum) were deployed as described in an international ring test for bioaccumulation of chemicals in earthworms [43], and 15 chickpea, 20 common wheat and 20 radish seeds were sown as proposed by OECD guidelines [44; 45], per soil column. Soil samples were taken for analysis of glyphosate and its major breakdown product AMPA (aminomethylphosphonic acid) allowing a 24-hour period for herbicide and its breakdown product to distribute along the soil column since it was not mixed with soil as in the case of LAS.

At day 14 three locusts were deployed per column; in one column per control and treatment in each cart, no locusts were deployed in order to evaluate plant growth and chemical concentration at the end of the experiment.

In the end of the experiment, day 28, aerial part of the plants was cut off and weighed; locusts were collected for survival assessment and the ones that survived were weighted and analysed for the presence of organic xenobiotics. The soil columns were removed from the cylinder and homogenised in order to take samples that were analyzed for humidity and LAS, glyphosate and AMPA. Earthworms were collected for survival assessment and the ones that survived were weighted and analysed for the presence of chemicals.

Chemical Analysis

Soil, plant and invertebrate samples were analyzed by terracon GmbH (http://www.terracon-jueterbog.de/) with HPLC-FLU/ELCD analogue DIN EN 38407-F22 in the case of Glyphosate and AMPA, and HPLC-UV acc. to internal method No.QA-TENS01/03 in the case of LAS.

Risk Assessment – scenarios and probabilistic assumptions

Since a site-specific risk assessment was intended, it was decided to use, as far as possible, realistic exposure levels of Roundup Ultra and LAS. In the case of the herbicide the application was made following the recommendation of Bayer Crop Science (http://www.bayercropscience.pt/) of a maximum agronomic application rate of 10 l ha^{-1} for pastures and wheat crops, which meant a volume of 24 µl per microcosm. The disposal of sewage sludge in agricultural soil in Castro Verde was preceded by chemical analysis but only metals were analyzed [26]. The present Portuguese regulation regarding the usage of sewage sludge in agriculture [46] foresees a LAS limit of 2600 mg kg^{-1}_{dw} in sludge but until 2002 when the program aiming to prevent desertification and soil erosion [26] ended, organic compounds were not covered by national [47] or EU legislation [48]. Considering the predicted environmental concentrations (PEC) in sludge amended soils, modelled in household uses from the LAS risk assessment by HERA [49] of 5.6 mg kg^{-1}, and the range of 1-10 mg kg^{-1} referred to as a worst-case scenario with a dosage of 2 T ha^{-1} in a Danish Workshop on LAS risk assessment [12], a concentration of 10 mg kg^{-1} was tested in microcosms, knowing that in Castro Verde a mass of 5-6 T ha^{-1} at depth of 30-75 cm was amended [26].

PEC values of glyphosate, AMPA and LAS for plants and invertebrates were obtained from the measured concentrations in microcosm testing. For the calculation of PECs in small mammals and in target birds, formulas were adapted from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [50]. The bioaccumulation factors of organics in small mammals was obtained from the formula $BAF = \alpha * F / k_2$, where α is the fraction of ingested dose that is absorbed, F is the food intake rate per body weight (FIR/bw) (calculations followed information provided in appendix I of the referred guidance document), and k_2 is the rate constant for depuration. In the case of glyphosate $\alpha = 0.3$ (30 %) and $k_2 = 1$ (100 % at the end of 168 h) [24; 51]; for AMPA toxicokinetics can be characterized by $\alpha = 0.2$ (20 %) and $k_2 = 1$ (100% between 24 and 120 h) [52]; and for LAS values are as follows $\alpha = 0.85$ (80-90 %) and $k_2 = 0.63$ (60-65 %) [49]. PEC calculations for the ecological receptors are given by the formula: $PEC_{Bird} = (FIR/bw) * C * PD$, where C is the concentration of organic chemical in fresh diet and PD is the fraction of food type in diet [53]. Diet of small mammals was assumed to include equal proportions of the tested plant species but for adult Great Bustards, diet was considered to be constituted on the proportions described by Palacios *et al.* [32] for the plant families tested in microcosms experiments: Fabaceae (40 %) for *C. arietinum*, Brassicaceae (32 %) for *R. sativus*, and Poaceae (28 %) for *T.*

aestivum. In view of the diet described for *C. pygargus* by Corbacho *et al.* [42], the following percentages of food were considered for the PEC calculations: 70 % herbivorous small mammals, 20 % insectivorous small mammals, and 10 % locusts.

Risk characterization is based on the comparison between exposure and assessment but the way this comparison is formally conducted differs depending on the considered protocols [54]. EU guidelines for the assessment of plant protection products (e.g. [53]) compare toxicological endpoints (depending on target organisms) with exposure according to a Toxicity to Exposure Ratio (TER = Toxicity/PEC). In the approach for industrial chemicals proposed by the EU Technical Guidance Document on Risk Assessment [55] the assessment of effects is based on the establishment of Predicted No Effect Concentrations (PNEC's) and characterization is given by a PEC/PNEC ratio. According to the EU's Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) [54] the main advantage of TER comparisons lies on the possibility of evaluating the ecological relevance of the identified potential risk. Since the present model and assessment scenarios already cover the ecological specificities of the different elements of the trophic chain, additionally allowing the differentiation between bird species and even within the same species (juvenile and adult Great Bustard), and in order to standardize risk assessment the simplified comparison such as the PEC/PNEC ratio was used. For the effects assessment, PNECs were derived from literature ecotoxicological data for active substances and following the principles of the Technical Guidance Document on Risk Assessment [55] for the application of safety (assessment) factors due to secondary poisoning. Hence, from a no observed effect concentration (NOEC) for reproductive toxicity to birds at a concentration of 200 mg kg^{-1} glyphosate (assessment factor of 30) [51] a PNEC of 6.67 mg kg^{-1} was derived. In relation to AMPA a no observed adverse effect level (NOAEL) for maternal and developmental toxicity of 400 mg kg^{-1} bw day^{-1} in rat (Olson 1991 cit. in [52]) was converted (conversion factor of 10) into a 4000 mg kg^{-1} NOEC (subchronic test; assessment factor of 90), giving a PNEC of 44.44 mg kg^{-1}. Regarding LAS, a PNEC of 5.56 mg kg^{-1} was derived from a NOAEL of 50 mg kg^{-1} bw day^{-1} in rat [49] converted (conversion factor of 10) into a 500 mg kg^{-1} NOEC (duration of the test 90 days; assessment factor of 90). For characterizing the risk of glyphosate, AMPA and LAS an additional safety factor of 10 was included for covering the individual variability within vertebrate species, as the assessment focuses on defined species with ecological value which should be protected at least at the population level within the area. For transparency reasons this factor was not included in the PNEC derivation, but in the interpretation of the risk values. Thus, the PNECs were developed following the TGD recommendations, but the acceptability threshold for the PEC/PNEC was established as 0.1, instead of 1 which is the value recommended in the guidance document for generic assessments.

The refinement of risk assessment was done using the probabilistic approach with Monte Carlo analysis (10000 trials) performed with Crystal Ball software [56]. As assumptions, the concentrations measured in plants and invertebrates were given a normal distribution, and PEC and risk characterization (PEC/PNEC ratio) for target birds were defined as forecasts.

Results

Exposure

Glyphosate and AMPA

As a result of the agronomic application rate of Roundup Ultra, at the end of a 24-hour period a concentration of 0.02 mg kg^{-1} of glyphosate could be found in soil (table 4.II). But at the end of 28 days, the concentration of herbicide was below the quantification limit of the analysis equipment (< 0.01 mg kg^{-1}). The concentration in invertebrates was also below the quantification limit (< 0.1 mg kg^{-1}) but in the case of plants the uptake was extremely high when comparing concentration in *C. arietinum*, *T. aestivum* and *R. sativus* with soil concentration. The breakdown of glyphosate could be observed after 24 hours since the concentration of AMPA at day 0 was two orders of magnitude higher (33.62 mg kg^{-1}). At the end of the experiment, AMPA was still detectable in soil and the uptake occurred in both plants and invertebrates.

Since only in plants the uptake of glyphosate could be determined by analytical methods, the calculation of PEC was only done for herbivorous small mammals and for adult Great Bustard that feed on plants. For Montagu's Harrier PEC was also calculated since they feed on herbivorous small mammals to which BAF was calculated. The birds' exposure to glyphosate is laid down in table 4.III with the deterministic approach whereas the probabilistic distributions are shown in figure 4.2. Both bird species bioconcentrate glyphosate but adult Great Bustard, that feed only on plants, accumulate higher concentrations of herbicide; Montagu's Harrier prey on herbivorous small mammals that have BAF lower that one, hence lower concentrations of glyphosate reach the top predator. As the result of the Monte Carlo analysis and following the normal distributions assumed in the PECs for plants, exposure in birds presents a normal distribution, but the range of concentrations in adult Great Bustard is higher that in Montagu's Harrier.

Given that for AMPA the uptake could be determined in plants and invertebrates, PEC was calculated for all birds, as shown in table 4.III for the deterministic approach and in figure 4.3 with probabilistic methods. Adult Great Bustard and Montagu's Harrier, that bioaccumulate glyphosate, have concentrations of AMPA less than 1 mg kg^{-1}. While not bioaccumulating the parent compound, juvenile Great Bustard and Lesser Kestrel show higher bioaccumulation of AMPA. This fact may be explained by its diet based on locusts that bioconcentrate AMPA (BAF of 2.25 kg kg^{-1}). These differences on birds' exposure can also be observed from the Monte Carlo analysis along a normal distribution that reflects the assumptions for exposure in plants and invertebrates.

Table 4.II. Chemical concentrations (wet weight) in soil and in the organisms from the microcosms' experiments. Glyphosate and its breakdown product AMPA were analyzed in soil samples at day 0 and day 28. Soil samples were analyzed at day -1 and day 28 for the presence of LAS.

GLYPHOSATE concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day 0	0.02	-	-	-	-	-
Day 28	< 0.01	< 0.1	1.71	2.17	0.79	< 0.1

AMPA concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day 0	33.62	-	-	-	-	-
Day 28	19.59	4.80	2.88	5.97	1.62	10.80

LAS concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day -1	10.13	-	-	-	-	-
Day 28	5.06	14.74	5.06	8.28	3.30	3.96

LAS

In the beginning of the experiment the concentration of LAS in soil was of 10.13 mg kg^{-1} but after 28 days it was reduced by half (table 4.II). Considering concentration in soil at day 28, bioconcentration took place in *E. andrei* and at a less extent in *R. sativus* and *C. arietinum*.

The different pathways consequential from the birds' diet results in PEC values of the same order of magnitude for all ecological receptors. This fact can be observed in both deterministic (table 4.III) and probabilistic approach (figure 4.4). Like in the Monte Carlo analysis performed for herbicide and its breakdown product, PEC values for LAS in target birds follow a normal distribution as a result of the assumptions for exposure in plants and invertebrates.

Table 4.III. Deterministic risk assessment of Glyphosate, AMPA and LAS for the target bird species: exposure assessment (PEC), effects assessment (PNEC) and risk characterization (PEC/PNEC).

	Glyphosate				
	Adult Great Bustard	Juvenile Great Bustard	Lesser Kestrel	Montagu's Harrier	
PEC	0.39	-	-	0.06	mg kg^{-1}
PNEC		6.67			
PEC/PNEC	0.58	-	-	0.09	

	AMPA				
	Adult Great Bustard	Juvenile Great Bustard	Lesser Kestrel	Montagu's Harrier	
PEC	0.84	4.42	2.83	0.42	mg kg^{-1}
PNEC		44.44			
PEC/PNEC	0.19	0.99	0.64	0.09	

	LAS				
	Adult Great Bustard	Juvenile Great Bustard	Lesser Kestrel	Montagu's Harrier	
PEC	1.35	1.62	1.04	1.82	mg kg^{-1}
PNEC		5.56			
PEC/PNEC	2.43	2.91	1.87	2.28	

Risk Characterization

In the deterministic approach for risk characterization point estimates are obtained and when the PEC/PNEC ratio is lower than 0.1, risk is considered acceptable since an additional safety factor of 10 was included for covering the variability within bird species. From table 4.III it may be assumed that the utilization of glyphosate at agronomic application rates: poses an acceptable risk for Montagu's Harrier and a low risk for adult Great Bustard, Lesser Kestrel and juvenile Great Bustard although, for the last two low risk characterization is due to the metabolite AMPA because they do not uptake the parent compound. The present tested LAS concentration in soil shows a scenario with risk for all the considered protected birds from the SPA of Castro Verde.

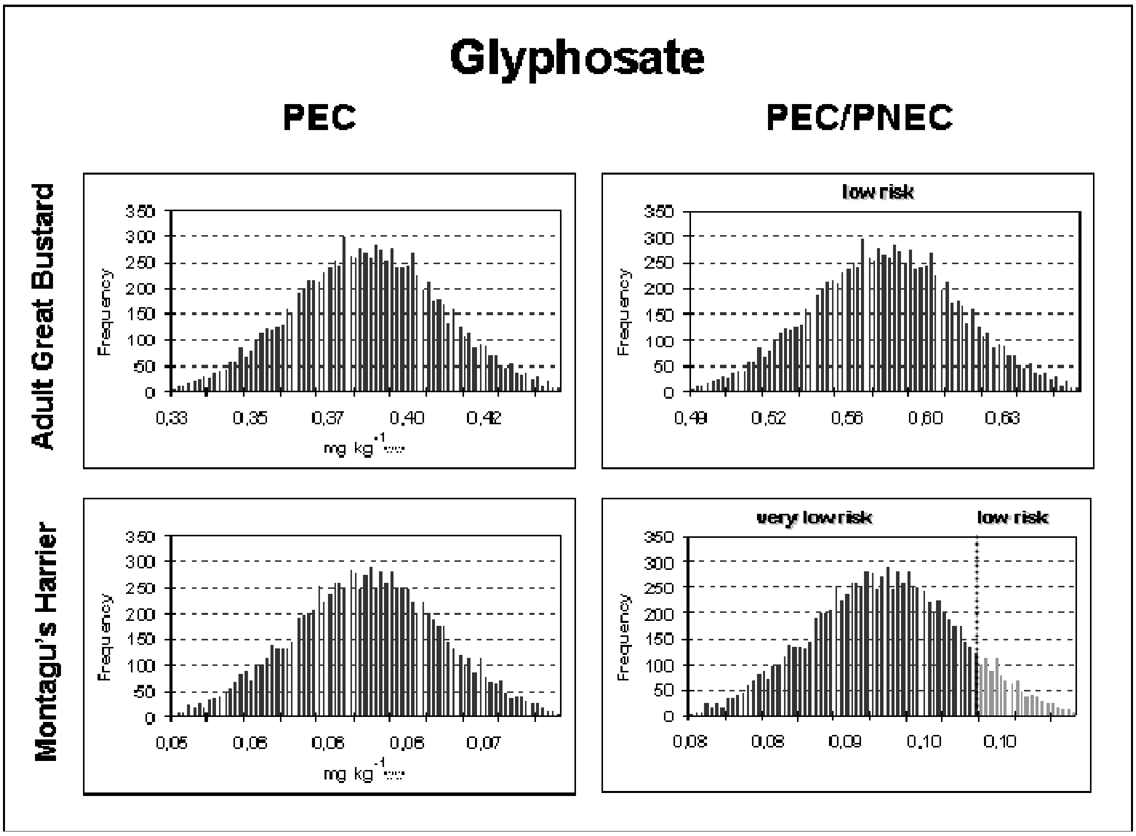


Figure 4.2. Probabilistic risk assessment of Glyphosate for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

The Monte Carlo analysis with Crystal Ball allows the forecast of the distribution of concentrations where the likelihood of the extent of a defined range may be quantified. Thus for the characterization of risk with the probabilistic approach four classes of risk were defined (figures 4.2, 4.3 and 4.4): (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. The risks from the usage of glyphosate, following the manufacturer suggested application rate, are considered to be: very low to Montagu's Harrier with a 20.94 % probability of low risk due to AMPA; low for adult Great Bustard and Lesser Kestrel; and low for juvenile Great Bustard but with a probability of 47.67 % of potential risk due to the degradation metabolite. The considered worst-case scenario for LAS concentration in sludge amended soils may pose a potential risk for birds of conservationist concern from the SPA of Castro Verde, namely *O. tarda*, *F. naumanni* and *C. pygargus*.

Discussion

For the assessment of glyphosate its degradation metabolite AMPA must also be taken into account and consequently when comparing the risk of the two compounds for the same bird, the higher level of risk must be considered. In the case of the Montagu's Harrier in spite of being the top predator hence expected to be exposed to higher concentrations of toxicants due to secondary poisoning through the food chain [54], glyphosate was assessed to be of acceptable risk or of an overall very low risk according to the probabilistic distribution. This fact may be explained by the low BAF calculated for small mammals (BAF in small herbivorous mammals: 0.26 glyphosate and 0.17 AMPA; BAF in small herbivorous mammals: 0.41 glyphosate and 0.27 AMPA) that constitute the main diet of this raptor [42]. The risk of glyphosate may be considered to be low for adult Great Bustard. Considering the unmetabolized parent glyphosate, there was no exposure, i.e. no contact between stressor and receptor, for juvenile Great Bustard and Lesser Kestrel since it was not bioaccumulated in their food items, i.e. locusts. But the breakdown product AMPA was accumulated along the food chain though posing low risk to *F. naumanni* but potential risk to juvenile *O. tarda* like the hawk feeds on locusts but has higher feeding rates per body weight. It is clear the advantage of assessing the risk by means of probabilistic methodologies since according to the determinist approach only a low risk could be observed for juvenile Great Bustard but the exposure distribution showed almost 50 % of probability of potential risk of glyphosate degradation products, despite being indicated as a low toxic substance to vertebrates [52]. The effects on juvenile individuals have consequences at the turnover of the population thus jeopardizing the conservation of the species.

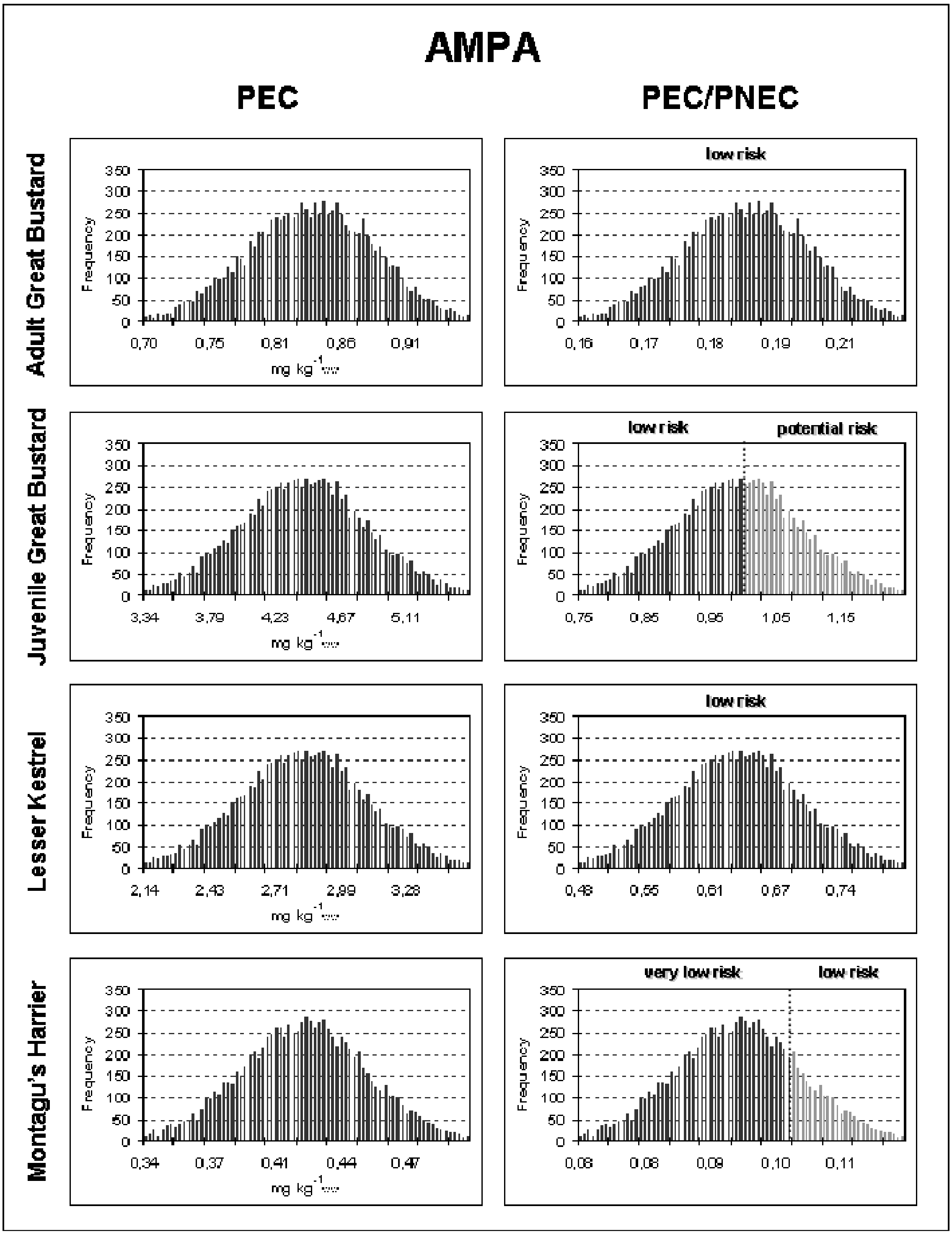


Figure 4.3. Probabilistic risk assessment of AMPA for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

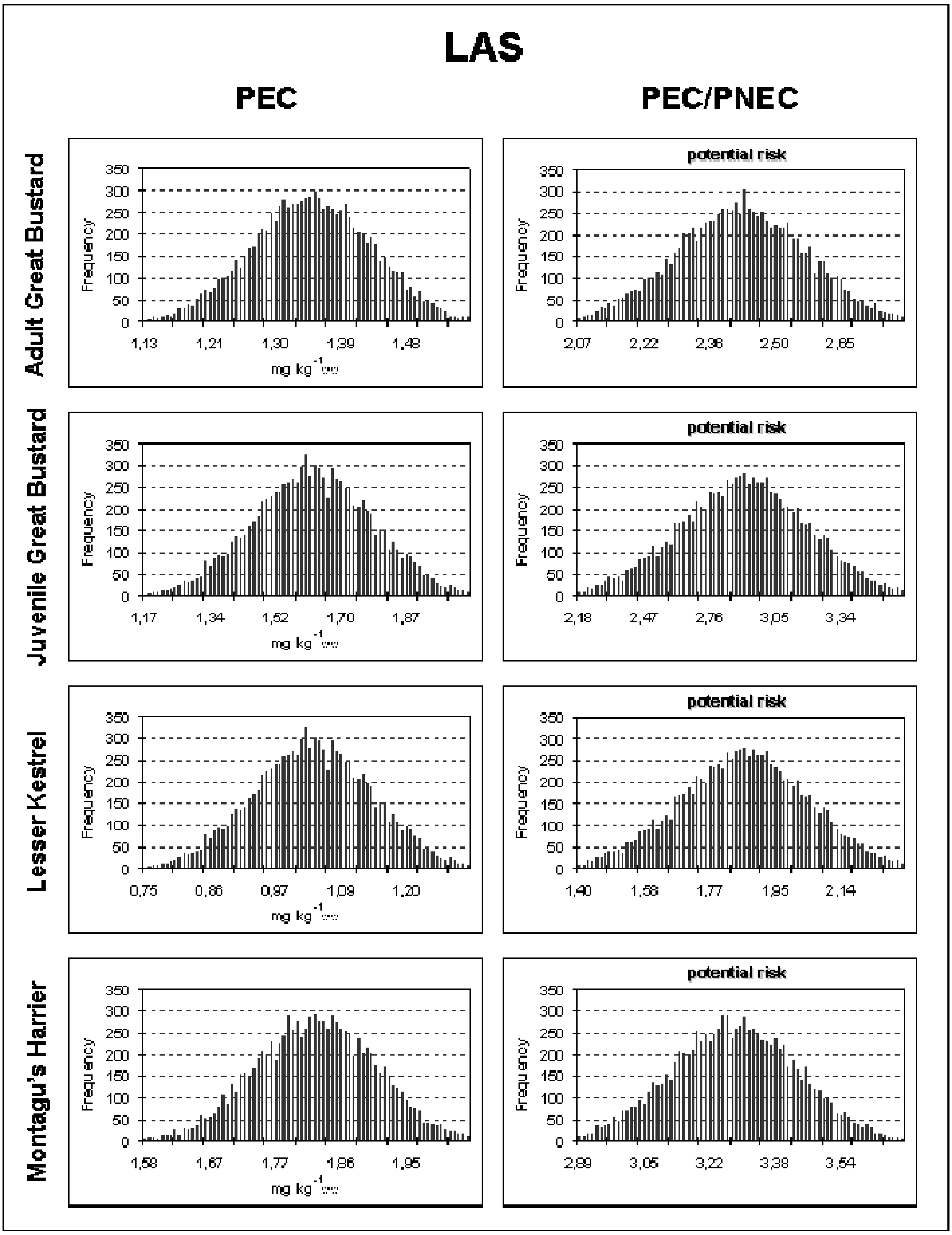


Figure 4.4. Probabilistic risk assessment of LAS for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

Some of the main facts referred in literature to explain why LAS is not dangerous to the environment are the evidence that it is readily degraded by aerobic microbial processes and does not bioaccumulate [57; 25; 49]. In fact some studies show that LAS is not even uptake by plants [12]. This may also be explained by the fact that it binds strongly to organic matter due to its negative charge [11]. Furthermore there is a common agreement on the fact that LAS has no risk for terrestrial (and aquatic) compartment as assessed by HERA [49] and the occurrence of bioconcentration is highly unlikely with an extremely low potential for secondary poisoning [58]. Nonetheless the present experimental work with terrestrial microcosms and respective risk assessment indicate that LAS may bioaccumulate in plants and concentrate through the food chain, and be responsible for secondary poisoning, having a potential risk for the considered bird target species, as assumed in our initial hypothesis. The fact that the soil from Castro Verde has low organic matter content may account for plants and invertebrates uptake hence influencing its transfer along the food chain. The uncertainty remains on whether the chosen scenario for exposure, 10 mg kg^{-1} of LAS in soil, might have been too high but organic compounds were not analysed in the sludge amended in Castro Verde nor were references found for background concentrations of LAS in Portuguese sludge-amended agricultural soils.

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CHAPTER 5

Chapter 5. The perception of risks from extensive agriculture in a Nature 2000 Network site

Abstract

Risk communication stands after risk assessment procedure, as a step where the exchange of information takes place between scientific community and stakeholders. Therefore the perception of risks to the different target groups needs to be studied. The present paper describes an evaluation of the perception of the risk, to general public, farmers and local authorities, from extensive farming practices in a cereal steppe in Castro Verde, southern Portugal (Alentejo). This area is included in the European Nature 2000 Network as a special protection area (SPA) for wild birds in accordance to the Birds Directive (79/409/EEC) due to its importance in the conservation of protected species like the Great Bustard (*Otis tarda*). For the purpose a questionnaire-based survey was carried out in the municipality of Castro Verde. The agriculture sector showed respondents with higher percentage of academic degree (but also a significant part of the respondents had only attended primary school) and a better knowledge on precise aspects of the SPA. The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. The environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information about the SPA to the people from the region .This work is an important contribute for the development of a risk communication framework for risks posed by extensive agriculture in a Natura 2000 Network site.

Keywords: risk communication, risk perception, natura 2000 network, extensive agriculture.

Introduction

There is frequently a gap in communication between the knowledge obtained by the scientific community and the general public, which delays the prevention and resolution of environmental problems. Risk Communication is defined by the OECD [1] as the interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public. Thus communication is an important tool in understanding environmental problems and in the orientation of decision-making [2]. But in order to decisions being understood and accepted by potentially affected individuals or groups as well as the general public it is important that risk communication takes into consideration perception of people towards environmental risks. In Europe, Risk Assessment protocols are used to set the impact of chemical contamination on biota [3]. This scientific step underpins the decision-making process defined as Risk Management that involves considerations of political, social, economic, and technical factors [1]. Therefore risk communication is fundamental in enhancing the likelihood that risk management decisions will incorporate the results of the risk assessment [4].

Questionnaire-based surveys have been used by European institutions to determine how people perceive risk, being therefore a contribution to the development of policy initiatives and communication events related to risk issues [5; 6]. A similar endpoint to "perception of risk" is used to deal with the loss of biodiversity; public awareness was defined as an indicator for biodiversity in the "Proposal for a first set of indicators to monitor progress in Europe" by the European Environmental Agency, [7]. Of course public awareness is not the same as public perception but ultimately both concepts are addressed when aiming to induce a cultural change towards sustainability.

In the EU, a network of protected areas, Natura 2000 Network is being built on the designation of areas for conservation under the Birds and Habitats directives, the so-called "nature directives", that constitute the European policy basis for halting the loss of biodiversity. Once in Natura 2000 Network the conservation status of habitats and species listed in the directives must to be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [8; 9]. One of the dominant land uses in the EU is farmland (arable land and permanent grassland) that covers more than 45 % of the territory. Traditional farming practises like extensive agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [10]. In Portugal more than 25 % of Natura 2000 habitats depend upon the continuation of extensive farming practices whereas the average EU-15 value in 2004 was of 18 % [11]

The key objective of the present work is to contribute for the development of a risk communication framework for risks posed by chemicals associated to extensive agriculture practice in a Natura 2000 Network site. Thus we aimed to assess the awareness and perception of the risk, to general public, farmers as the major actors in the continuation of extensive agricultural practices, and local authorities and decision-makers.

Methodology

A questionnaire-based survey was performed in the Municipality of Castro Verde in southern Portugal, Alentejo, since it has the higher percentage of land (55 %) from a Special Protection Area (SPA) for wild birds with a total area of 79007 ha. This questionnaire aimed 3 different target groups: general population, people from the agriculture sector and servants from local authorities. The survey will provide information for a risk communication process subsequent to the assessment of risks posed to the ecosystem by extensive agriculture within the limits of the SPA.

Study area

The SPA of Castro Verde is characterized by extensive farm fields with no arboreal vegetation and some less representative habitats with no agricultural use such as shrublands (of scrub *Cister ladanifer*) and woodlands (mainly holm oak *Quercus rotundifolia* but also a few olive groves *Olea europea*). This Mediterranean cereal steppe habitat is the refuge for some bird species of conservationist concern like the Great Bustard (*Otis tarda*) [12]. The traditional soil use creates a landscape mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [13; 14].

The Questionnaire

The survey aimed to get a general picture of perceptions and views among the different stakeholders from the SPA of Castro Verde. Knowing that respondents would have different cultural and professional backgrounds, the questions selected tried to be as unambiguous and simple as possible, and an overall effort was made to make the questionnaire absolutely understandable.

After a brief introduction and invitation to participate, a group of questions were set to assess the socio-demographics of the respondents (Annex 5). The questions that followed were based on a previous survey conducted within an ongoing project on Global Sustainability Assessment in a Spanish SPA (Ramos M. J., personal communication) with a similar habitat and populations of birds from the same species. Firstly we wanted to know how far people were informed about the SPA, e.g. its ecological values, and who provided them with that information. Finally it was intended to gain inside on what people think about the factors that underpin the conservation of the SPA, and in the last question respondents were asked to reflect about the risks of a series of hazardous activities.

Target groups

It was decided not to carry out conventional in-person interviews. Instead, everyone whom the questionnaire was distributed was asked to fill it on their own to avoid any kind of disturbance or influence due to the presence of the people who carried out the survey. The square root of the population set our objective for the total number of respondents; since the population of the Municipality of Castro Verde is of 7603 people, ninety people were interviewed.

For the general public group (N = 36), participants were recruited among pedestrians and business establishments in the centre of Castro Verde village. The preferential targets were adults but some teenagers also participated in the survey.

In order to obtain as much people from the agricultural sector as possible the survey was performed during the period of the call to the Zonal Program of Castro Verde that was held in the “Campo Branco” Farmers’ Association. Contiguous to the office where the call was going on there is the store of the “Campo Branco” Farmers’ Association that sells several agrochemicals and veterinary medicinal products. Therefore people from both places were recruited (N = 31), that included, every kind of jobs related to agriculture and livestock farming, as well as land owners that may had other occupations not related to farming at all.

Servants from local authorities (N = 23) were recruited directly from their working places, namely the municipality hall of Castro Verde, and the parish offices at Castro Verde, Casével, Santa Bárbara de Padrões and São Marcos da Atabueira. Respondents were selected at random from different sections and working posts reaching people from different professional and educational backgrounds.

Results and discussion

The response rate was not measured but, except for local authorities, it can be estimated that only ca half of the people that were addressed accepted to fill the questionnaire. From our interpretation of people's excuses for not participating, it seems that they were afraid of being evaluated on they answered to the questionnaire and in many cases people had difficulties in reading and interpreting the survey.

Socio-demographics

The socio-demographic characterization of the respondents is given in table 5.I. Whereas for general population the proportion of men and women was almost the same, in the agricultural sector men were overrepresented (69 %), and in local authorities women were in larger number (65 %). People that accepted to participate among the pedestrians and business establishments were largely under forty years old (72 %). In the agricultural sector the majority lied in the range of 40-65 years old (55 %) but a significant percentage of people were under this range and older than 25 years old (35 %). Public servants from the municipality and parishes that participated were, of course in working age, mainly in the range of 30-50 years old (70 %). For all groups respondents inhabited mostly in Castro Verde village (parish), because except for the parish offices in the other villages, the survey was carried out there. People that lived in other municipalities were also considered for the survey because their professional activity was developed in the municipality of Castro Verde or because their municipality of residence was also included in the SPA.

Table 5.I. Socio-demographic characterization of the respondents. (Values are given in terms of percentage.)

	General public (n = 36)	Agriculture sector (n = 31)	Local authorities (n = 23)
Gender			
Male	47.2	69.0	34.8
Female	52.8	31.0	65.2
Age			
<18	13.9	0.0	0.0
19-24	8.3	6.9	4.3
25-30	13.9	20.7	17.4
31-40	36.1	13.8	26.1
41-50	11.1	31.0	43.5
51-65	13.9	24.1	8.7
>66	2.8	3.4	0.0
Education			
Illiterate	2.8	0.0	0.0
Basic – 1st degree	5.6	23.3	4.3
Basic – 2nd degree	8.3	6.7	4.3
Secondary – 1st degree	41.7	20.0	43.5
Secondary – 2nd degree	36.1	13.3	17.4
Academic degree	5.6	36.7	30.4
Occupation			
Student	16.7	0.0	0.0
Unemployed	5.6	0.0	0.0
Public servant	2.8	0.0	100.0
Farmer	2.8	53.3	0.0
Self-employed worker	11.1	16.7	0.0
Employee/ Worker	58.3	30.0	0.0
Retired	2.8	0.0	0.0
Municipality and Parish			
Castro Verde			
Castro Verde	81.8	62.1	60.9
Casével	0.0	6.9	8.7
Sta. Bárbara de Padrões	3.0	0.0	8.7
S. Marcos da Atabueira	0.0	3.4	4.3
Another Municipality	15.2	27.6	17.4

Most of the respondents from the general public group received 9 years (secondary first degree, 41 %) or even 12 years of education (secondary second degree, 36 %). One of the respondents in spite of being illiterate was able to read and fill the questionnaire with crosses. 58 % of the respondents were employed or working and this was the only group where students participated in the survey. The agricultural sector had the higher percentage of people with a university degree (37 %) but within this group the second highest level of education represented was the first degree (primary school) (23 %). This shows that this group consisted of two kinds of people: with higher education (engineers or veterinarians) that worked in the sector or were the land owners; and workers that performed tasks with a lower level of education (e.g. truck drivers or land workers) or that managed smaller agriculture fields. Respondents from local authorities were quite well educated, with 39 % of people with a university degree, but most of the servants had nine years of education (secondary first degree, 44 %).

The SPA

First of all, it was possible to know how respondents thought about the level of information they possessed on the SPA of Castro Verde (figure 5.1). On a scale of zero to five it was coincident that the mode for the responses was of three (followed by two). This does not clarify particularly the question as it is the medium level of the scale. But if we look at the extremes, zero and one for not informed at all, and four and five for very well informed, and the overall trend, a better outlook on the differences of opinions between groups is obtained.

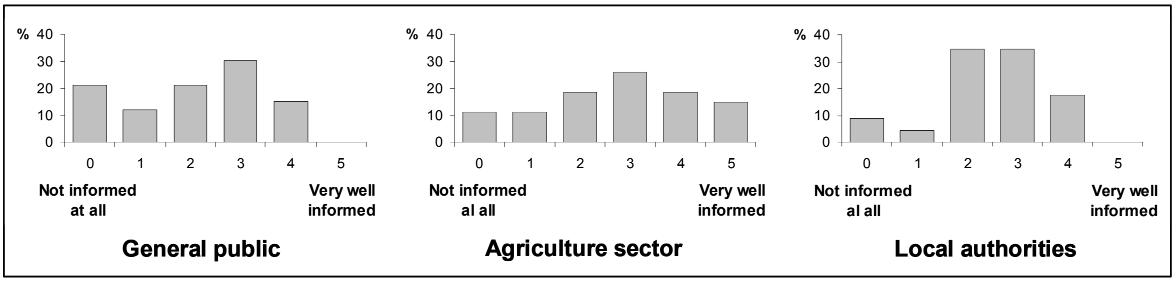

Figure 5.1. Respondents' opinion on the level of information they possess on the SPA of Castro Verde, from 0 as not informed at all to 5 as very well informed.

General public indicated the lowest level of information, with the highest percentage of respondents with reduced information level (33 %). Local authorities represented the group with least variability, indicating an average level of information, with just 13 % indicating a reduced level of information and no one assuming to be very well informed. The opposite was observed for the agriculture sector, where 22 % indicated a low level of information, while 33% considered themselves to be well or even very well informed. These results indicate that the group that dealt directly with the management of the SPA (agriculture sector), by carrying out directly their professional activities included a subgroup which considered itself to be knowledgeable in terms of information on the SPA characteristics.

In terms of precise aspects of the SPA the agricultural sector also presented a better insight as can be observed in the graphs of figures 5.2 and 5.3.

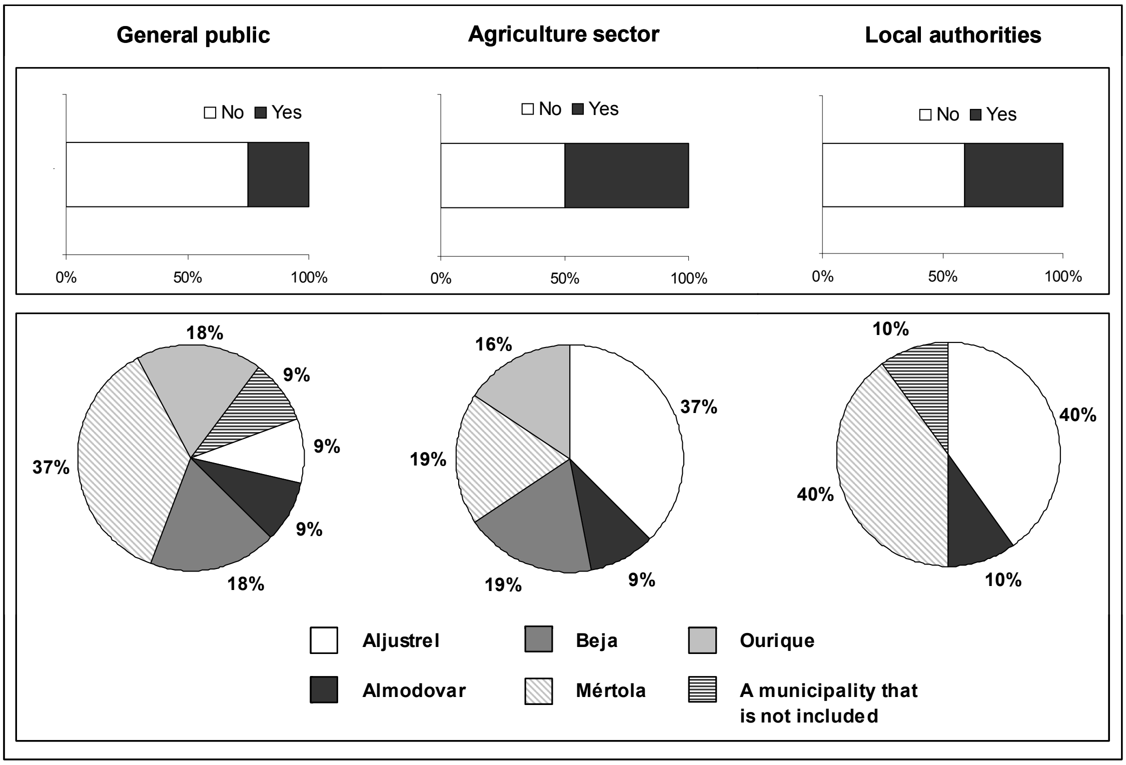

Figure 5.2. Besides Castro Verde, do the respondents know another municipality included in the SPA?

50 % of the respondents from the agriculture group knew at least one municipality included in the SPA, other than Castro Verde (figure 5.2). The municipalities also included are Aljustrel, Almodovar, Beja, Mértola, and Ourique, and all of them were referred by this group. From the general public, only 25 % new another municipality from the SPA, but 9 % of the respondents indicated one that is not included. 59 % of the public servants that participated new another municipality, but 10 % indicated a municipality that has not part in the SPA, and only two other municipalities (Almodovar e Mértola) were referred.

According to the Portuguese Institute for Nature Conservation [12] the SPA of Castro Verde in southern Portugal, Alentejo, is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*) that is one of the most important labels of that region [15]. But there are also other species of conservation concern and that is why respondents were asked if they knew other birds besides the Great Bustard (figure 5.3). The majority of people from the agriculture and local authorities groups knew other protected species (respectively, 63 % and 61 %), namely the Stone Curlew (*Burhinus oedicnemus*), the White Strock (*Ciconia ciconia*), the Black-bellied Sandgrouse (*Pterocles orientalis*), the Common Crane (*Grus grus*), the Lesser Kestrel (*Falco naumanni*), the Woodchat (*Lanius* sp.), the Little Bustard (*Tetrax tetrax*), and the Montagu's Harrier (*Circus Pygargus*). From the general public, less than half of the respondents (46 %) stated to know other bird species. The species that were referred to more often in all groups were the Black-bellied Sandgrouse, the Lesser Kestrel and the Little Bustard. Among the local authorities' respondents, the Lesser Kestrel corresponded to 76 % of the references. This is not surprising since two months before the survey took place a book on the Lesser Kestrel was presented [16] with the cooperation of the Municipality of Castro Verde. This event may also have contributed to the fact that a larger number of public servants got to know an additional protected species from the SPA. Another curiosity is the fact that people from the agriculture sector indicated six bird species whereas the other groups referred to only five.

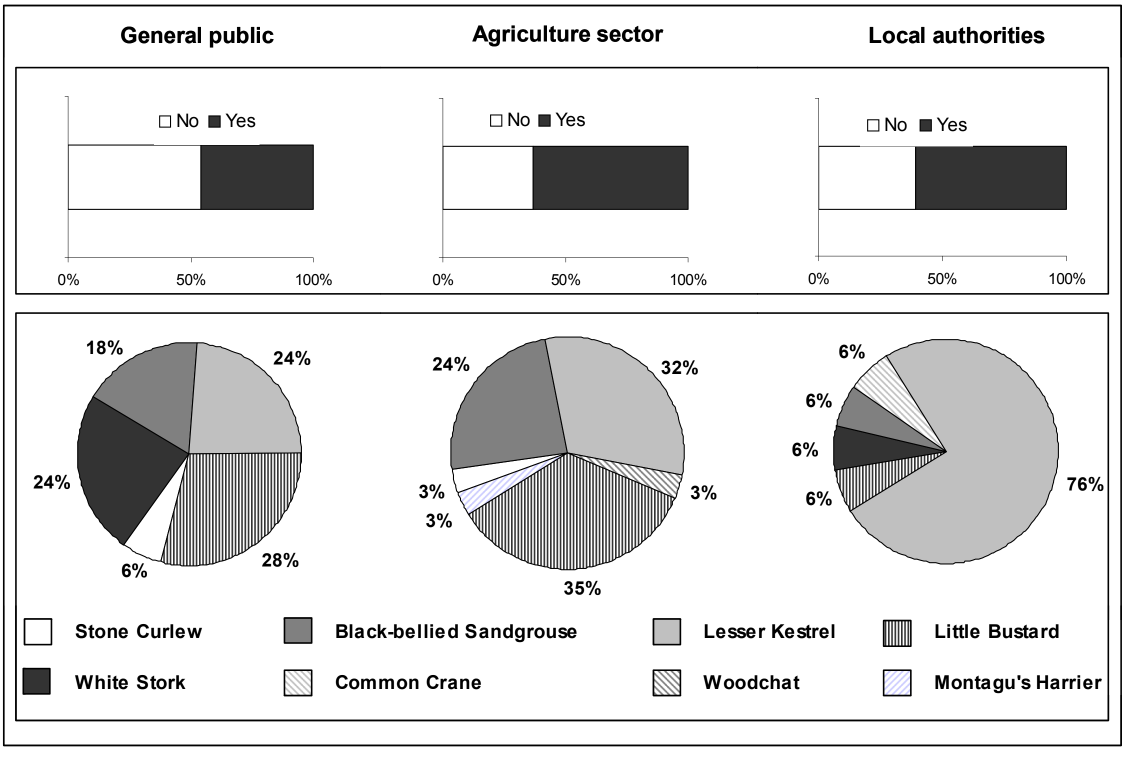

Figure 5.3. Besides the Great Bustard, do the respondents know another protected species in the SPA?

In a statement, how do people feel about the SPA? Is it an advantage or a disadvantage for the Municipality and the different human activities that may take place there? The majority of the respondents (90 % of the local authorities and 85 % of the general public) thought the SPA was a natural resource for educational, environmental and ecotourism purposes (figure 5.4). But a significant percentage of the agriculture sector (30 %) saw it as a surplus-value for agriculture and valorisation of the region, and 7 % considered the SPA a restriction for agriculture and local development. This sector experiences the direct consequences or any possible restrictions that may outcome from the classification of the site under the Natura 2000 Network. That is why the subject was a little bit more controversial, what makes it more surprising that 15 % – a higher value than the general public and this statement is not even considered by local authorities – of respondents claimed not to have an opinion on the subject.

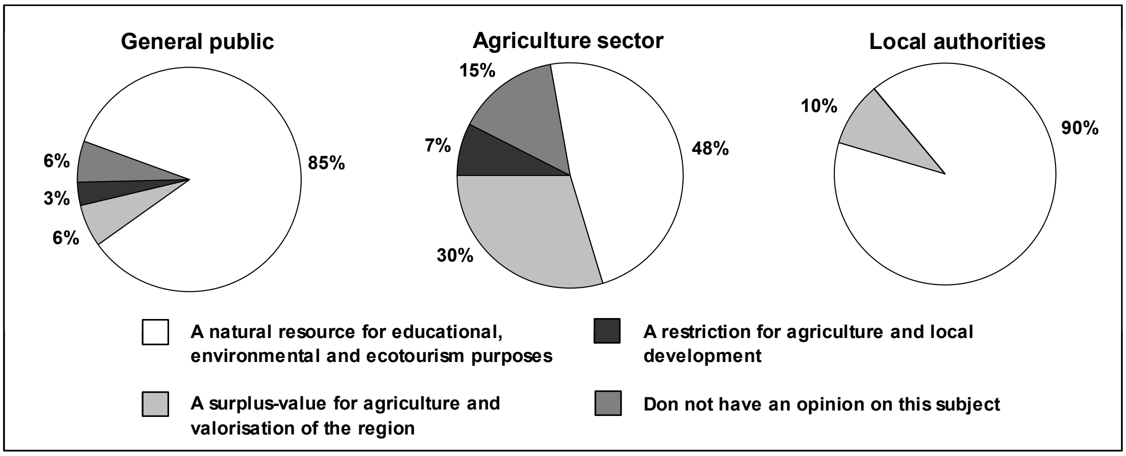

Figure 5.4. What do the respondents think of the SPA for Castro Verde and the other included municipalities?

The overall positive attitude towards the SPA among the inhabitants of the municipality of Castro Verde denotes a clear sensitivity towards the ecological value of this site, even to people that are directly affected from the management restrictions in a Natura 2000 Network. In a contingent valuation survey in Portugal, a significant part of the interviewed people (59.9 % web based and 45.7 % in-person) were willing to pay to preserve the Cereal Steppe o Castro Verde as an annual governmental tax and as a voluntary contribution) [17]. Both results show a considerable sensitivity of population in relation to services ecosystems and the environment may provide. By informing people about the SPA there is a large probability that an even greater understanding and perception of the ecological values will develop. But what are the sources of information from the Natura 2000 Network site of Castro Verde (figure 5.5)?

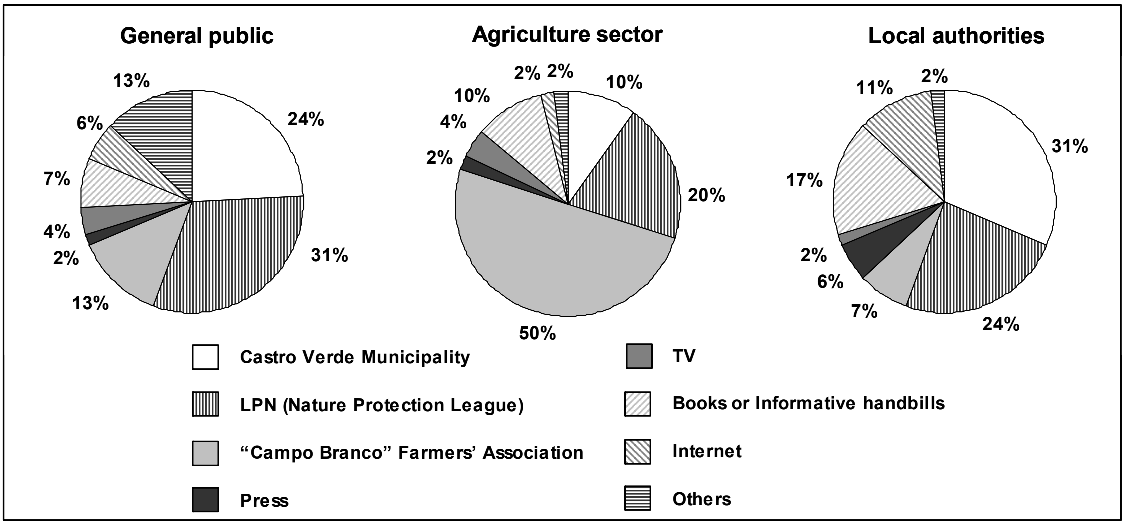

Figure 5.5. What was the source of information respondents have on the SPA for Castro Verde?

Not surprisingly, most of the respondents from the agriculture sector (50 %) stated their knowledge on the SPA of Castro Verde came from the "Campo Branco" Farmers' Association and public servants (31 %) stated it was provided by the Municipality of Castro Verde. Looking at all target groups the environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information. This NGO has been developing in the region, since the early nineties, a sustainable model for agriculture, involving local farmers, and integrating it with several scientific, touristic and environmental projects. Press, television, internet and books or informative handbills, are also important sources of information to respondents.

SPA Conservation

The main habitat for steppic birds is created by extensive farming with fallow [18]. Thus the generality of species are favoured by open landfills. But how far do people understand that the maintenance of this habitat is important for the conservation of the SPA? Many may think that by planting trees, specially the autochthonous species like the Cork Oak or the Holm Oak a more favourable habitat to the generality of birds may be created. The majority of the respondents (87 %) from the agriculture sector and local authorities understood the importance of the maintenance of extensive agriculture, but almost half of the general public that participated in the survey did not know what the most important factor for the conservation of the SPA of Castro Verde was (table 5.II).

Table 5.II. Analysis of the respondents' opinion on the factors that underpin the conservation of the SPA of Castro Verde.

	General public (n = 36)	Agriculture sector (n = 31)	Local authorities (n = 23)
What is the most important factor for the conservation of the SPA of Castro Verde?			
Maintenance of extensive agriculture with cereal fields and fallow	44.4	86.7	87.0
Increase in Cork Oak and Holm Oak forest landscapes	13.9	3.3	4.3
Do not know	41.7	10.0	8.7
Do you think the traditional agricultural practices are only possible by being supported with agro-environmental funding (Zonal Program)?			
Yes	47.2	93.3	60.9
No	52.8	6.7	39.1

Since 1995 such agricultural scheme is being supported by agri-environmental measures' under the Zonal Program of Castro Verde. Reviewed in 2003 [19], this program allows financial compensation to farmers who voluntary agree to maintain the traditional agricultural system with the cereal-fallow rotation, in an area larger than one hectare. It is an important tool to overcome the fact that as a low intensity dry cereal farming land it represents a marginal economic system with a yield of 14 % of the EU average [18]. As can be observed from table 5.II the importance of the Zonal Program was absolutely clear for the agriculture sector (93 %), since the economic viability of their activity is dependent in the financial support. Whereas for local authorities the importance of this funding was also clear (61 %), but more than half of the general public (53 %) did not think so.

Risks to the SPA

The main threats for the SPA of Castro Verde and probable risks for the steppic bird species have been identified [15; 12; 18; 16]: intensification of agriculture and livestock farming – with increasing of agrochemicals input, and installation of fences and land irrigation systems – on one side, or abandonment of agriculture on the other; the forestation with Eucalyptus or Pine trees due to rather advantageous EC funding; and illegal hunting and death by electrocution when colliding with electric cables, especially to Great Bustard populations. Other less conspicuous inputs of toxic chemicals in the ecosystem are sewage sludge and livestock manure. In recent years in Castro Verde, wastewater sludge was used as fertilizer in a program aiming to prevent desertification and soil erosion. Previous studies under Mediterranean climatic conditions have showed the fertilization with sewage sludge may pose risk to soil invertebrates [20; 21]. As for livestock, toxic concentrations of veterinary medicinal products that can be found in dung and urine [22] may contaminate soil due to grazing or when manure is used as fertilizer in agriculture.

A final question was drawn in the survey so that respondents could reflect and assess a series of risks that might affect the SPA (figure 5.6). Albeit the fact that in table 5.II it was clear respondents perceived the importance of the maintenance of extensive farming, a reduced percentage of answers assessed the intensification of agriculture (and development of systems for land irrigation) with a high risk for the SPA. Many considered it as moderate risk, but this intermediate assessment was frequent in almost every factor that was presented. The issues that were considered with a higher risk by all target groups were agriculture abandonment, herbicides, illegal hunting and, at some extent, death by electrocution and the disposal of sewage sludge as soil amendment. The factors considered to pose a lower risk were the ones related to livestock – cattle increase and usage of manure as fertilizer in agriculture.

When comparing the assessments performed by the different target groups there were not any major differences. Still in some issues agriculture sector showed some small variations in the perception of risks. It was the group with a higher percentage of high risk assessments to intensification of agriculture (32 %, followed by the general public with 21 %), as the economy of this group has a direct relationship with the SPA activities it might be expected them to wish to turn farming as profitable as possible. However, the overall assessment must also consider their higher level of knowledge as well as the socioeconomic correction provided by the agro-environmental funding. Moreover, they looked to the risk of forestation with more careful, and 24 % of the respondents considered it as high. As for herbicides, they were looked at with less concern, when compared with the other target groups; 50 % of the respondents considered the risk of the utilization of herbicides as being high when this assessment was of more than 80 % in the other groups. There was also less concern towards illegal hunting (56 % high risk, 36 % moderate risk) in comparison with public and local authorities (ca 80 % high risk, ca 20 % moderate risk).

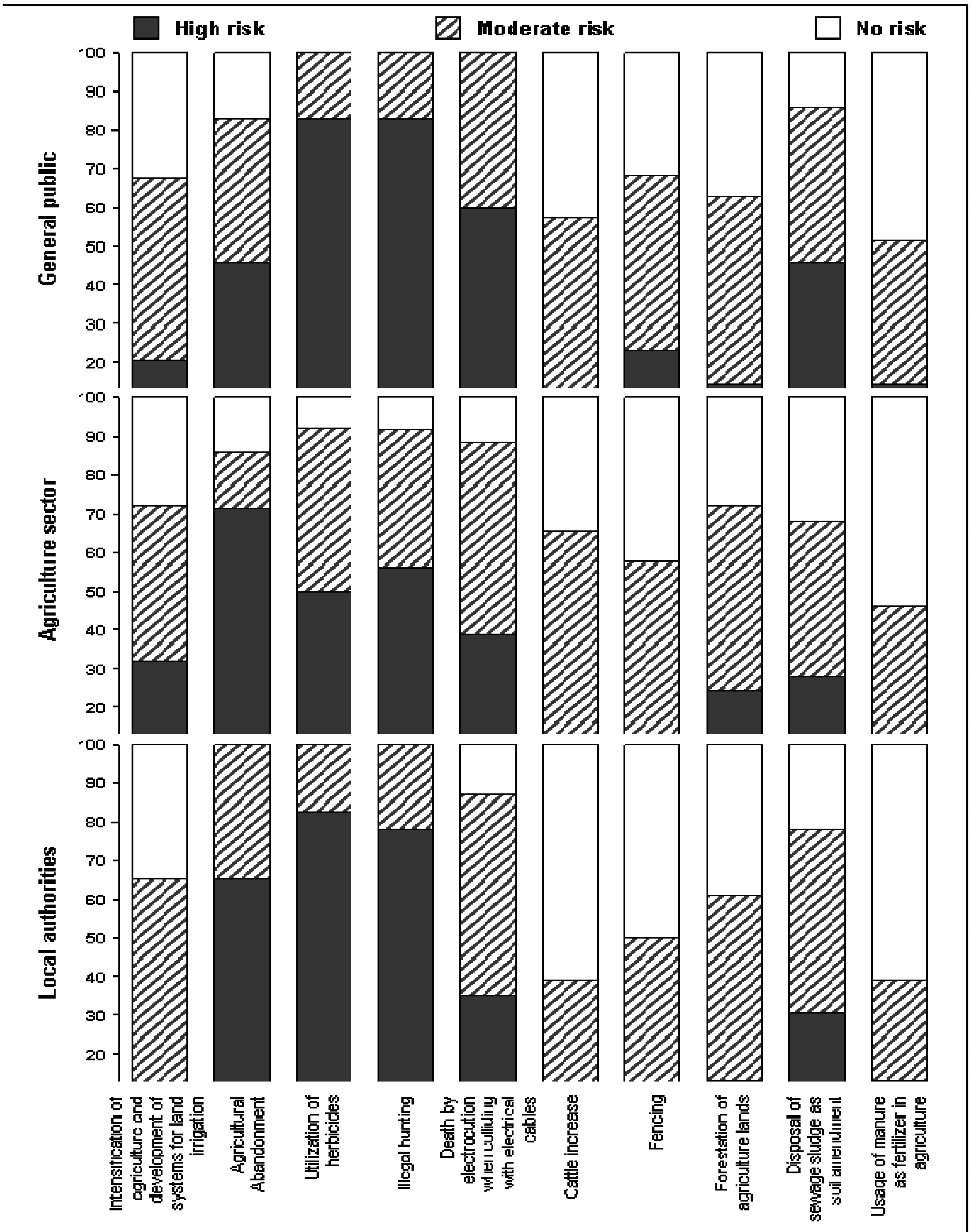

Figure 5.6. Respondents' opinion on risks affecting the SPA for Castro Verde.

Conclusion

Following a risk assessment in the SPA of Castro Verde and knowing the diverse perceptions and characteristics of the different stakeholder it is possible to develop tools for an effective risk communication framework, important in the environmental governance of this Natura 2000 Network site.

First of all in order to decide on the amount of information provided and how it is deployed, one must be aware of the cultural background of the population. And the only objective way of doing so is by the analysis of their educational background. The majority of the population has received at least more than 9 years of education (secondary first degree) and in the local authorities this proportion exceeds 90 %, having 30 % of the public servants an academic degree. The agriculture sector is the target group with a higher percentage of people that attended university (37 %) but a significant percentage of the group (23 %) has only been at primary school. Therefore the heterogeneity of the agriculture sector must be taken into account when preparing communication events.

The agriculture sector presented the better knowledge on precise aspects of the SPA – municipalities that are included in its limits and protected bird species – and a higher percentage of respondents considering themselves very well informed. Most of the people from general public and local authorities consider the SPA a natural resource for educational, environmental and ecotourism purposes but in the case of the agriculture sector it is also seen as a surplus-value for agriculture and valorisation of the region. The "Campo Branco" Farmers' Association and the Municipality of Castro Verde were considered the most important sources of information for, respectively, the agriculture sector and for public servants but the NGO, LPN, has shown to have an extremely relevant role in informing all target groups about the SPA of Castro Verde.

The importance of the maintenance of extensive agriculture of cereals with fallow rotation, supported by agri-environmental measures, is understood by local authorities and especially to the agriculture sector that relies economically in this scheme, but general public does not share this perception.

The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. They seem to disregard the intensification of agriculture, maybe because they do not link it, along with the development of systems for land irrigation, with the destruction of extensive cereal steppe habitats.

There are several potential factors that could contribute to this assessment such as knowledge, heritage, and socio-economy. The higher level of information on the environmental values of the SPA, perceived by the agriculture group and also confirmed by the answer to relevant questions, may indicate a greater capacity for perceiving certain risks, in particular the relationship between conservation and traditional extensive farming. Additionally, the cultural heritage, and the clear perception of agri-environmental funding as an essential need for maintaining sustainable agricultural practices, and last but not least, the fact that this funding has been available for years, should be considered. It is important to mention that only 7% of the enquired persons within this group perceived the SPA measures as limitations for agricultural development, while 30% perceived the opposite, considering the SPA as an opportunity for local valorisation. The Common Agriculture Policy of the EU is considered a key tool for socioeconomic balance among regions. The aims and objectives have been adapted and current measures focus mostly on the protection of the environment and the farmers' quality of life. In a global market, sustainable rural development is demonstrating a clear capacity as alternative to intensive agriculture. Issues such as food quality, food safety, food diversity, animal welfare, are more and more appreciated by the European citizens. The added value gained by these issues may compensate the final yield economic balance. Additional opportunities related to leisure activities such as ecotourism, and recognising the role of extensive agricultural in the SPA as a service provided by the farmers to the society (biodiversity conservation) that should be compensated, are also relevant when interpreting the differences in the perception among the three groups. Although the questionnaire does not allow a formal interpretation, a significant issue identified within this study is the role of source for information. The group related to the agricultural sector had received the information basically through an agricultural organization and an environmental NGO, the combined information seems to offer in general a proper level of knowledge on the ecological and socioeconomic implications of the SPA.

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Annex 5. Survey on the SPA of Castro Verde

We are undergoing a study in an area of high ecological value of Castro Verde designated as "SPA of Castro Verde", and we would like your help by answering the following survey questions in order to us to know how informed you are on this special protection area. This information will be taken into consideration in future actions.

THANK YOU VERY MUCH ON YOUR COOPERATION!

Gender: male ☐ female ☐

Age: _____

Education: ____

Occupation ____

Parish and Municipality of residence: __________________________

SPA: SPECIAL PROTECTION AREA FOR WILD BIRDS

How informed are you about the SPA of Castro Verde? (from 0 as not informed at all to 5 as very well informed)

0	1	2	3	4	5

Besides Castro Verde, do you know another municipality included is this SPA?

YES		NO	

In case you said yes, indicate one: ______________________________

Do you know any protected species of this SPA besides the Great Bustard?

YES		NO	

Indicate one: ________________________

What do you think of the SPA for Castro Verde and the other included municipalities? (indicate one option only)

- A natural resource for educational, environmental and ecotourism purposes ☐
- A natural space with no advantage or usefulness at all ☐
- A surplus-value for agriculture and valorisation of the region ☐
- A restriction for agriculture and local development ☐
- Don not have an opinion on this subject ☐

What was the source of the information you actually have on the SPA of Castro Verde (indicate one or more options):

- Castro Verde municipality ☐
- LPN (Nature Protection League) ☐
- "Campo Branco" Farmers' Association ☐
- Press ☐
- TV ☐
- Books or Informative handbills ☐
- Internet ☐
- Others ☐
  Which ____

What is the most important factor for the conservation of the SPA of Castro Verde?

- Maintenace of extensive agriculture with ceral fields and fallow ☐
- Increase in cork oak and holm oak forest landscapes ☐
- No not know ☐

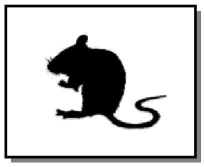
Do you think the traditional agricultural practices are only possible by being supported with agro-environmental funding (Zonal Program)?

YES		NO	

Assess the following agents according to your personal degree of acceptability towards the risk for the wild birds at the SPA of Castro Verde:

	HIGH RISK	MODERATE RISK	NO RISK
▪ Intensification of agriculture and development of systems for land irrigation			
▪ Agricultural Abandonment			
▪ Utilization of herbicides			
▪ Illegal hunting			
▪ Death by electrocution when colliding with electrical cables			
▪ Cattle increase			
▪ Fencing			
▪ Forestation of agriculture lands			
▪ Disposal of sewage sludge as soil amendment			
▪ Usage of manure as fertilizer in agriculture			

CHAPTER 6



Chapter 6. General Discussion

Risk Characterization

Given our hypothesis, due to the biomagnification of chemical pollutants along the food chain it would be expected the occurrence of secondary poisoning of protected birds in the Special Protection Area (SPA) of Castro Verde: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). Hazard of herbicide glyphosate [1-3], and linear alkylbenzene sulphonate (LAS) [4-8] and cadmium [9-13] present in sewage sludge used for soil amendment had been identified in literature. Inclusively, in one hand secondary poisoning had been demonstrated previously [14-16] and contamination of birds due to metals [17; 18] and pesticides is well documented [19; 20]. On the other hand the Montagu's Harrier exhibits a positive mean population trend in Europe [21], and the populations of Great Bustard and Lesser Kestrel have been recently increasing in the SPA of Castro Verde [22; 23]. But one of the major threats to this SPA, and to high nature value farmlands in general across the EU, is the intensification of agriculture [24; 25] with deleterious consequences to biodiversity, namely birds [26]. Therefore assessing real- and worst-case scenarios for the exposure of toxic chemicals will give us a predictive in-sight on agriculture intensification and also on the repeated exposure effects to bird communities due to present extensive agricultural practises.

For the study of the interactions between trophic levels and determining the transfer of chemicals through the trophic chain terrestrial microcosms were used, and predicted environmental concentrations (PECs) and bioaccumulation factors (BAFs) were obtained. Experimental data allowed us to calculate values for plant and invertebrate species important in the ecological receptors' food chain. Plant species important to the agricultural scheme of the SPA of Castro Verde were chosen in accordance to *O. tarda* feeding preferences: common wheat (*Triticum aestivum*), chickpea (*Cicer arietinum*), and cultivated radish (*Raphanus sativus*) [27-29]. Earthworms *Eisenia andrei*, were tested as key elements involved in secondary poisoning of organisms that feed upon them [13], and due to the role they play in water infiltration and storage and soil aeration [30] thus contributing for the mobilization of metals [31]. Locusts *Schistocerca gregaria* were selected since Orthoptera are important food items for juvenile *O. tarda* [28] but also to *F. naumanni* [32] and, to a less extent, to *C. pygargus* [33]. BAF and PEC values for small mammals and PEC values for target bird species were calculated adapting the formulas from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [34]. Predicted no effect concentrations were derived from literature toxicity data following the principles of the Technical Guidance Document on Risk Assessment [35]. An additional safety factor of 10 was included to cover for the individual variability within bird species, as the assessment focuses on defined species with ecological value which should be protected at least at the population level within the area. For transparency reasons this factor was not included in the PNEC derivation, but it was used in the interpretation of the risk values. Finally, for the characterization of risk a standardized approach for all chemicals was performed on the simplified comparison of the PEC/PNEC ratio. The refinement of risk assessment was done using the probabilistic approach with Monte Carlo analysis (10000 trials) performed with Crystal Ball software [36]. The characterization of risk with the probabilistic approach allowed us to distinguish four classes of risk: (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. Calculations for probabilistic risk assessment under a realistic scenario are presented in table 6.I.

Table 6.I. Probabilistic assessment of risks posed to birds of conservationist concern from the SPA of Castro Verde under a real-case scenario.

	Herbicide	Sewage Sludge	
	Glyphosate	Cd	LAS
Adult Great Bustard	100 % low risk	100 % low risk	100 % potential risk
Juvenile Great Bustard	52 % low risk 48 % potential risk	39 % low risk 61 % potential risk	100 % potential risk
Lesser Kestrel	100 % low risk	93 % low risk 7 % potential risk	100 % potential risk
Montagu's Harrier	79 % very low risk 21 % low risk	1 % low risk 99 % potential risk	100 % potential risk

Herbicide usage

The uptake of glyphosate that resulted from agricultural application rate could only be determined by analytical methods for plants and therefore PEC was calculated only in the case of adult Great Bustard and Montagu's Harrier. However, for the assessment of glyphosate its degradation metabolite AMPA must also be taken into account and consequently when comparing the risk of the two compounds for the same bird, the higher level of risk must be considered. The overall risk for Montagu's Harrier is very low, with a 20.94 % probability of low risk due to AMPA. The pathway for herbicide in *C. pygargus* food chain is affected by the low BAF values of small rodents (herbivorous mammals), the most important item of its diet, thus explaining the low uptake and concomitant low risk. The risk of glyphosate may be considered to be low for the adult Great Bustard. Considering the unmetabolized parent glyphosate, there was no exposure, i.e. no contact between stressor and receptor, for juvenile Great Bustard and Lesser Kestrel since it was not bioaccumulated in their food items, i.e. locusts. But the breakdown product AMPA was accumulated along the food chain though posing low risk to *F. naumanni* but ca. 50 % of potential risk to juvenile *O. tarda* despite being indicated as a low toxic substance to vertebrates [2]. The effects on juvenile individuals have consequences at the turnover of the population thus jeopardizing the conservation of the species.

Sewage sludge amendment

The risk from Cd and LAS present in sludge-amended soils must be assessed in separate scenarios since when sewage sludge was added to soil in Castro Verde, only metals were analysed in accordance to the Sewage Sludge Directive 86/278/EEC [37] (and national legislation, n.º 118/2006 [38]).

In the case of Cd, several scenarios were considered but microcosm experiments were performed for a worst-case considering the concentration in sewage sludge; hence BAF calculations were made with PEC in soil from this scenario. A more realistic scenario was assessed with the estimated soil concentration dependent exclusively from sewage sludge amendment but the real-case scenario comes from adding to this PEC the Cd from baseline concentrations in agricultural soil from Castro Verde, that exactly matches the PEC for a generic Regional environment (PECregional) calculated in the Risk Assessment Report [13]. In this scenario risks from Cd are low for adult Great Bustard, but there is a 7 % probability for Lesser Kestrel, a 61 % probability for juvenile Great Bustard and even a 99 % probability for Montagu's Harrier, of potential risk from the amendment of sewage sludge. Thus the top predator *C. pygargus* has the most critical food chain for secondary poisoning. Another scenario of concern was the one of the maximum level of Cd permitted by the Sewage Sludge Directive 86/278/EEC [37] (and national legislation, n.º 118/2006 [38]) to be added to agricultural land per year, that even disregarding the baseline Cd concentration showed a potential risk to Montagu's Harrier of 56 %.

PEC for LAS was derived from soil concentrations in Denmark in a worst-case scenario with a sludge dosage of 2 T ha^{-1}. But according to the information regarding the usage of sludge in Castro Verde, a mass of 5-6 T ha^{-1} at depth of 30-75 cm was amended [39], probably turning the worst-case Danish PEC in a realistic exposure for our case-study. There is a common agreement on the fact that LAS has no risk for terrestrial (and aquatic) compartment as assessed by HERA [8] and the occurrence of bioconcentration is highly unlikely with an extremely low potential for secondary poisoning [7]. Nonetheless the present experimental work with terrestrial microcosms and respective risk assessment indicate that LAS may bioaccumulate in plants and concentrate through the food chain, and be responsible for secondary poisoning, having a potential risk for the considered bird target species, as assumed in our initial hypothesis. The fact that the soil from Castro Verde has low organic matter content may account for plants and invertebrates uptake hence influencing its transfer along the food chain.

Risk Communication

The perception of the ecological values and risks from extensive agriculture to different stakeholders – general public, agriculture sector and local authorities –, in the SPA of Castro Verde was assessed with a questionnaire-based survey, as part of the risk communication process. The agriculture sector presented the better knowledge on precise aspects of the SPA and a higher percentage of respondents considering themselves very well informed. The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. They seem to disregard the intensification of agriculture, maybe because they do not link it, along with the development of systems for land irrigation, with the destruction of extensive cereal steppe habitats and the input of chemical toxicants. The environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information about the SPA to the people from the region.

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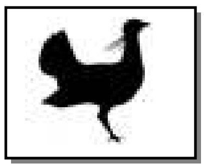
Thesis Structure

The present thesis will be structured in six chapters. In the first chapter, the basic principles and concepts underpinning this work will be drawn in a General Introduction followed by the description of the Objectives with the rationale and scope of the thesis. In chapters two to five the work will be described in detail in the form of four Manuscripts that will be later on submitted to relevant SCI journals. Finally the major achievements of the thesis will be discussed in the General Discussion chapter.

CHAPTER 1



Chapter 1. General Introduction and Objectives

Biodiversity in Europe

One of the outcomes of the UN Earth Summit held at Rio de Janeiro (Brazil) in 1992 was the Convention on Biological Diversity (CBD) [1], where "biological diversity" was defined as *the variability among living organisms from all sources including, among others, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems*. The services healthy ecosystems with high biodiversity may deliver to mankind, often at no cost, have been pointed out by the Millennium Ecosystem Assessment (MA) [2] namely production of food, fuel, fibre and medicines, regulation of water, air and climate, maintenance of soil fertility, or cycling of nutrients. But MA has also shown that in recent times, especially over the past fifty years, the decline of biodiversity and respective changes in ecosystem services have been taking place, mostly due to habitat change, climate change, invasive alien species, overexploitation, and pollution. Needless to say human activities have been increasingly accentuating these deleterious drivers. Moreover changes in species diversity affect the ability of ecosystems to recover from disturbances, and thus underpin the resilience of ecosystems as well the services they provide [3].

According to the World Conservation Union (IUCN) [4], 147 vertebrate (mammals, birds, reptiles, amphibians and fish) and 310 invertebrate (crustaceans, insects and molluscs) species that occur in Europe are considered to be globally threatened, therefore categorised as critically endangered, endangered, or vulnerable. Albeit these numbers there were several significant commitments made by the EU regarding biodiversity along with many protection policies as part of the European strategy to conserve its critical wildlife and habitats. One important milestone was the signature of the above mentioned UN CBD where countries from the UN, EU countries included, recognized the biodiversity loss and its significance to society. In 1998 the EC Biodiversity Conservation Strategy was launched providing a comprehensive response to the many requirements of the CBD. Later on in 2001 the EU Heads of State or Government agreed to halt the decline of biodiversity by 2010 in the Göteborg European Council. One year later 130 world leaders, including the EU's, agreed to significantly reduce the rate of biodiversity loss by 2010 in the Plan of Implementation from the Johannesburg 2002 World Summit for Sustainable Development. At an important stakeholder conference held under the Irish Presidency of the European Council in Malahide in 2004, a broad consensus was achieved on priority objectives and a set of biodiversity indicators towards meeting the 2010 commitments, expressed in the "Message from Malahide". These biodiversity indicators were based on the first set of indicators adopted globally earlier in 2004 at the CBD 7th Conference of the Parties in Kuala Lumpur. By 2005 EU established a Streamlining European 2010 Biodiversity Indicators, where 26 indicators were proposed for different focal areas of biodiversity, including public awareness. Recently, in 2006 with an EC Communication on halting the loss of biodiversity, the extent of biodiversity loss was outlined but the adequacy of the EU response so far was also reviewed. In respect to the EU Biodiversity policy the basis for action is provided by the Birds and the Habitats Directives (the so-called "nature directives"). The strategic framework for the Commission's environmental policy is set by the Environment Action Programmes of the EC. The Sixth Action Programme for 2002-2012 [5] frames "Nature and Biodiversity" with the other environment priority areas and promotes full integration of environmental protection requirements into all Community policies and actions and provides the environmental component of the Community's strategy for sustainable development.

The Mediterranean Ecoregion

The highest number of plant and animal species in Europe is hosted in the Mediterranean basin, which has been identified by Conservation International as one of the world's 34 biodiversity hotspots [6]. The Mediterranean Hotspot surrounds the Mediterranean Sea and stretches west to east from Portugal to Jordan and north to south from northern Italy to Morocco, also including parts of Spain, France, the Balkan states, Greece, Turkey, Syria, Lebanon, Israel, Egypt, Libya, Tunisia and Algeria, with a total extent of more than two million square kilometres (figure 1.1). Islands from the Mediterranean Sea and from the Atlantic Sea – the Macaronesian Islands of the Canaries, Madeira, the Selvages (Selvagens), the Azores, and Cape Verde – are also part of this hotspot [6]. Physical background diversity is settled by numerous mountains as high as

4000 meters, peninsulas, islands and archipelagos. The bimodal weather pattern, that provides the unity to this ecoregion, is dominated by hot, dry summers, and cool, wet winters, with average annual rainfall ranging from less than 100 millimetres in desert territories to more than 4000 millimetres on certain costal massifs. In the western Mediterranean, the Iberian Peninsula, for at least two months each year there is frequently no precipitation al all, and most plants and animals experience a water deficit thus having developed ecophysiological or behavioural adaptations [7]. All this variety contributes to a high proportion of ecologically valuable areas and exceptional concentrations of biodiversity with 22500 species of vascular plants, nearly 500 bird species, more than 220 terrestrial mammals, more than 225 reptile species and nearly 80 amphibians, of which can be counted, respectively, 11700, 25, 25, 80, and 30 endemic species [6].

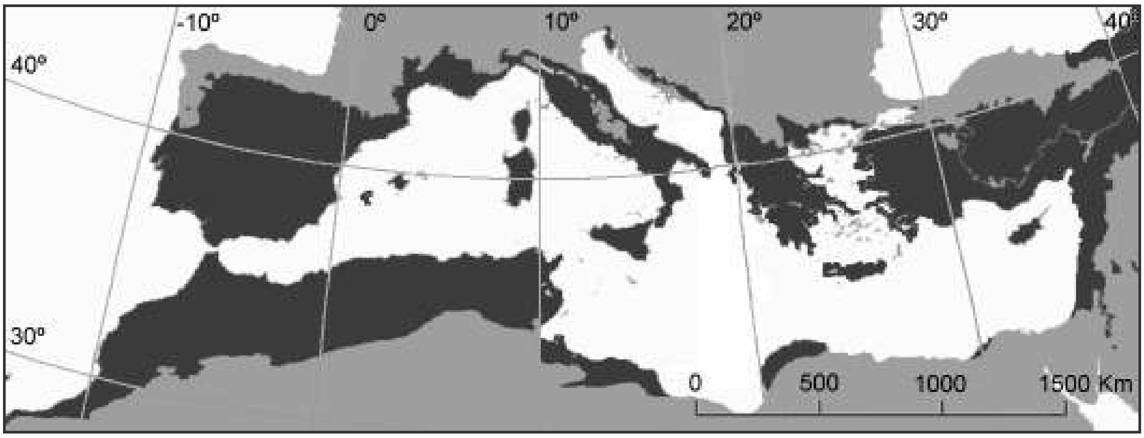


Figure 1.1. The Mediterranean Basin Hotspot. The dark shadows indicate the hotspot regions. Although Macaronesian Islands of the Canaries, Madeira, the Selvages (Selvagens), the Azores, and Cape Verde are not included in the map, they are part of the hotspot.
Adapted from the European Environment Agency Maps and Graphs data service [8] with information from the Biodiversity Hotspots webpage [6].

Agriculture and Biodiversity

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. Less than a fifth of the European land can be regarded as not directly managed. And of course biodiversity includes both managed and unmanaged ecosystems. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [9].

Following the overall trend, biodiversity in Europe's farmland has declined strongly in the last decades with a special emphasis to bird populations [10]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [11]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems. On the other hand the intensification of agriculture and concomitant increase in nutrient and pesticide inputs (chemical inputs will be further discussed in this chapter), generally leads to the decrease of biodiversity. Another factor that may jeopardise biodiversity of HNV farmland is agriculture abandonment as the result of low productivity that drive the socio-economic conditions of in rural areas unfavourable [12]. The Common Agriculture Policy (CAP) whilst being considered responsible for loss of biodiversity in rural areas by supporting greater productivity and consequently leading to agriculture intensification the [13], its agri-environment schemes – that exist since 1992 but became compulsory since the 2003 CAP reform – are important as funding instruments for promoting pro-diversity measures [12]. Therefore agriculture may be looked at not only for food production but also in the perspective of providing environmental services.

Protected Areas in Europe

Protected areas are fundamental policy tools for biodiversity and ecosystems conservation, especially for sensitive habitats [2].

The IUCN defines 6 categories of protected areas, depending on the management objectives, that are implemented in a network of 83 States [14]: Ia Strict Nature Reserve, Ib Wilderness Area, II National Park, III Natural Monument, IV Habitat/Species Management Area, V Protected Landscape/Seascape, and VI Managed Resource Protected Area. But at the EU level a network of protected areas, Natura 2000 Network, is being built on the designation of areas for conservation under the EU Birds and Habitats directives. Endangered and rare birds at the European or global level were firstly addressed by the Birds Directive [15], but this piece of legislation was afterwards complemented by the Habitats Directive [16] where habitats and other wildlife species were also considered. Thus Member States have designated Special Protected Areas (SPAs) for wild birds and then proposed Sites of Community Interest (SCIs) for habitats and endangered species, that encompass the Natura 2000 Network. In December 2006 it already covered more than 20 % of EU-25 territory [17]. Once in Natura 2000 Network the conservation status of habitats and species listed in the nature directives must to be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [15; 16].

SPA of Castro Verde

In Portugal more than 25 % of Natura 2000 habitats depend upon the continuation of extensive farming practices – that sustain HNV farmland – whereas the average EU-15 value in 2004 was of 18 % [11].

Figure 1.2. Municipalities within the SPA of Castro Verde. Adapted from the Natural Patrimony Information System of the Portuguese Institute for Nature Conservation [19].

Steppic areas are typical examples of HNV farmland from southern Europe with bird assemblages of conservation concern [12]. According to the Portuguese Institute for Nature

Conservation [18] the SPA of Castro Verde (figure 1.2) in southern Portugal, Alentejo, is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu's Harrier (*Circus pygargus*).

Classified under national law by the "Decreto-Lei nº 284-B/99" in September 1999, the SPA of Castro Verde includes six municipalities of which the municipality of Castro Verde has the higher percentage of land, 55 %, out of a total area of 79007.17 ha. In the last forty years average annual temperature from this region was approximately 22 ºC and annual rainfall 500-600 mm. For the same time span, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [20].

The main habitat of this SPA is characterized by extensive farm fields with no arboreal vegetation and some less representative habitats with no agricultural use such as shrublands (of scrub *Cister ladanifer*) and woodlands (mainly holm oak *Quercus rotundifolia* but also a few olive groves *Olea europea*). The overall scheme of farming is based on the following traditional rotation system: 1st year, primary cereal (wheat *Triticum aestivum*) – 2nd year, secondary cereal (oat *Avena sativa*) – 3rd year, fallow – 4th year, fallow – 5th year, land ploughed to reinitiate the cycle [18]. Cereals are usually sown in September-November and harvested in June-July [21] and leguminous crops (e.g. chickpea *Cicer arietinum*) are also sown in smaller amounts in summer. The main changes on this scheme occur on the duration of the fallow that is dependent on the fertility of the fields. Historically, livestock farming is based on extensive sheep production but nowadays cattle production is rapidly growing [18]. All these activities result in a landscape mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [22; 23]. The origin of this extensive agricultural system lies in the agricultural rush (Wheat Campaign) that took place in the 1930s as an attempt to make the country self-sufficient in wheat production. As a result of clearing of all existing vegetation (trees included) and ploughing of all types of soil, soil erosion was accelerated and the early intensive cereal cultivation system gave place to the present extensive agricultural pattern and livestock farming [24]. This is an area with the generality of soils that are poor and unsuitable for agriculture, where 75 % of the agricultural area is in fallow or works as permanent pasture land. Thus a low intensity non-irrigated cereal farming land it represents a marginal economic system with a yield of 14 % of the EU average [25].

The main threats to the SPA of Castro Verde are identified and result from the dual process of the abandonment of the less fertile agricultural soils with the intensification of agriculture in the remaining land. The result will most certainly lead to the impoverishment of this refuge for steppic birds due to: possible forestation because of the rather advantageous EC funding; increasing in cattle production, disappearance of the traditional cereal-fallow rotation, and installation of fences and land irrigation systems [18; 25]. Furthermore an input of chemicals in the ecosystem will tend to increase as it will be explained later on. One of the ways to overcome this hazardous trend is the financial support designated in this SPA as the Zonal Program of Castro Verde (ZPCV). These agri-environmental measures' management objectives are settled in national law, annexe I of "Portaria nº 1212/2003". Although the ZPCV was implemented in 1995, it was reviewed by the referred piece of legislation in October 2003 [18]. Shortly, if allows financial compensation to farmers who voluntary agree to maintain the traditional agricultural system with the cereal-fallow rotation, in an area larger than one hectare.

Birds in Castro Verde's SPA

Farmland birds are considered indicators for biodiversity because they are dependent on the ecological structure of agricultural habitats [12; 11; 3]. Since the 1970s it has been taking place an overall decline in farmland bird populations across Europe, a declining trend that is not apparent in bird assemblages of other habitats. This long-term trend suggests that the driver factors are specific to this habitat [10] and in fact agriculture intensification may account for the decline of more than 40 % of the bird species [12]. But as evidenced by Donald *et al.* [10] not all farmland species exhibited patterns of population decline. And if in the case of Great Bustard and the Lesser Kestrel there was a negative trend of, respectively, -1.1 and -1.39, other birds like the Montagu's Harrier exhibited positive mean trends (0.64).

Great Bustard (Otis tarda)

The Great Bustard (*Otis tarda*), family *Otididae*, is one of the largest birds of Europe and one of the heaviest flying birds of the world, typical of the steppic habitats and open lands with non-intensive farming [26]. Its populations though widely distributed, from the Iberian Peninsula to eastern Asia, are generally separated and consist of a few tens to several hundred individuals [27]. In Portugal there are estimated to exist 1150 individuals 80 % of which inhabit the SPA of Castro Verde. *O. tarda* is listed in the annex I of the Birds Directive [15] as well as categorized as VULNERABLE in the 2007 IUCN Red List [28] and in the Portuguese Vertebrate Red List [29].

This species has an accentuated sexual dimorphism with males weighting around 16 kg, which is 2 to 4 times the weigh of females (3-4 kg) [30], and measuring up to 1 m being therefore ca 50 % bigger than females [28]. The Great Bustard is a gregarious bird that lives in flocks with a variable number (4-16) of individuals around the year [30]. Feeding is the most time-consuming activity for the *O. tarda* [31]. They are omnivorous birds that feed mainly upon green plant material, and arthropods and seeds to a lesser extent [26; 27]. In fact green plant material accounts for 71 % in summer and autumn, and 95 % in spring and winter [32].

Lesser Kestrel (Falco naumanni)

The migratory Lesser Kestrel (*Falco naumanni*), Family *Falconidae*, has a Mediterranean range for breeding, heading south to Africa in winter, particularly to the southern Sahara region. It forages steppic habitats and grasslands with non-intensive cultivation [33]. In spite of being considered an endangered species all over Europe [34; 35], in Portugal it has been developing an increasing trend since 2001 (289 couples) until 2006 (445 couples), being the SPA of Castro Verde the territory for 73 % of the Portuguese breeding population [36]. *F. naumanni* is listed in the annex I of the Birds Directive [15] as well as categorized as VULNERABLE in the 2007 IUCN Red List [33] and in the Portuguese Vertebrate Red List [29].

This small hawk measures ca 30 cm and seldom exceeds 200 g of weight. Its rusty plumage bears him the right camouflage for the arid habitats where it lives [36; 33]. The Lesser Kestrel is a gregarious raptor that feeds mainly upon insects and preying activity tends occur in the surroundings of the colonies [37].

Montagu's Harrier (Circus pygargus)

The Montagu's Harrier, Family *Accipitridae*, has a widespread but patchy breeding distribution in Europe, which constitutes over 50% of its global breeding range [38], and it winters sub-tropical Africa and India, and around the Mediterranean Sea [39]. Although it is originally a marsh harrier it colonized the great extent of farmland that covers Europe [40]. The Portuguese population is the third largest in Europe with 900-1200 couples, being the breeding group in the SPA of Castro Verde the largest in the country [41]. *C. pygargus* is listed in the annex I of the Birds Directive [15], and tough considered of LEAST CONCERN in the 2007 IUCN Red List [38] it is categorized as VULNERABLE in the Portuguese Vertebrate Red List [29].

Being the smallest within the Harriers, this species measures 43-47 cm [39] and weights around 345 g although males tend to increase in body size as one goes west and south in Europe [40]. It is frequent to observe the reunion of couples in colonies while breeding showing therefore gregarious behaviour [41]. The Montagu's Harrier diet includes small mammals, mainly rodents, and occasionally small birds and large insects [42].

Toxic inputs in the SPA of Castro Verde

The present agricultural and livestock activities going on and the future trends of the SPA of Castro Verde make possible the input of several chemicals in this area of conservationist concern.

Herbicides

The herbicide glyphosate is marketed as a non-selective, broad-spectrum, post-emergence herbicide and is applied in this farmland area before seedling. It is a widely popular herbicide known for its effective control of competing vegetation, rapid inactivation in soil, and supposedly low toxicity to terrestrial invertebrates and mammals [43; 44]. But in fact it has been reported to affect the survival of earthworms [45], exert hepatic toxic effects to small rodents [46], and to affect not targeted plants in adjacent habitats to cultivated fields

[47], and therefore may threaten the wildlife vertebrates that rely upon these communities as food items.

Wastewater Sludge

In recent years in Castro Verde, wastewater sludge were used as fertilizers in a program aiming to prevent desertification and soil erosion [48] but these products were not assessed for the risks to the ecosystem in spite of the performed chemical analysis. Previous studies on the risks of the use of sewage sludge as fertilizer to soil microarthropod populations under Mediterranean climatic conditions revealed an impoverishment of the community structure and decrease in the diversity of Acari [49; 50]. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [51]. Moreover, problems with disposal of the accumulating sewage sludge in the municipal plants will probably lead to a compulsory use of these products as soil fertilizers. The presence of metals in the sludge is a clear concern and is regulated by the Sewage Sludge Directive 86/278/EEC [52]. Cadmium can be used as a model metal. According to risk assessment report of Cadmium (Cd) edited by the European Chemicals Bureaus [53] sewage sludge is a minor source of Cd for soils on an average basis; but it is a major source of Cd in soils where sludge is applied. However this risk assessment report does not assess the risks of Cd on soils where sludge is applied although it describes the potential hazards of Cd to soil fauna and plants from the revision of several research papers. Recently, the concern on the presence of micropollutants in the sludge has been extended to organic chemicals [54]. A large list of chemicals used in consumer products can be found in the sludge. Detergent components are of special concern in countries such as Denmark [55]. Linear alkylbenzene sulphonates (LAS) are the most widely used anionic surfactants in cleaners and detergents and are a major organic contaminant present in sewage sludge [56]. Terrestrial animals are not likely to be affected by sewage sludge LAS [55; 57] but its repeated addition needs to be assessed [58].

Veterinary Medicinal Products

The existence of livestock grazing in the area creates a less conspicuous pathway for the input of high local concentrations of toxicants into the ecosystem due to the veterinary medicinal products that can be found in the livestock dung and urine [59]. In the case of Castro Verde, the intensification livestock farming of cattle will most certainly become a vector for this type of contamination in a near future if not already present. Veterinary medicines are important safeguarding the health and welfare of livestock [60] but may have a potential impact in terrestrial ecosystems [61-64].

Europe's chemicals policy

According to the UN Programme of Action from the Earth Summit of Rio de Janeiro 1992, Agenda 21, improved risk assessment is necessary for the safe use of toxic chemicals (Section II, Chapter 19) [65]: "*Thousand of chemicals are used in every aspect of human endeavour but the long-term health and environmental risks of most of them are unknown. 95 % chemical manufacturing involves only 1500 chemicals but crucial data for risk assessment are lacking for many of them.*" In Europe the utilization of chemicals by human activities is regulated by several pieces of legislation implemented through guidance documents that foresee risk assessment protocols as the tool to set the impact of chemical contamination on biota [66]. With the introduction of the REACH, Regulation (EC) No 1907/2006 [67], risk assessment processes for existing substances will be further regarded and hasten.

For instance, if we consider the main probable toxic inputs in the SPA of Castro Verde, herbicides and sewage sludge, an assessment of their risks may be based in appropriate European guidelines. In the Annex VI of the Directive 91/414/EEC the detailed evaluation and decision making criteria for plant protection products (e.g. herbicides) is described [68]. Additional technical guidance is presented in Guidance Documents [69; 70] and in the outputs of the recent European Food Safety Authority scientific workshop on the revision of a guidance document on assessment of pesticide risks for birds and mammals [71]. Data for the risk assessment of sewage sludge may be found in the EC Directive 86/278/EEC on the use of sewage sludge in agriculture [52], namely the limit values for heavy metals, but limits for organic compounds are not included. The limit values for compounds such as LAS can only be found in the EC Working Document on Sludge [72] on the revision of the Directive 86/278/EEC. Nonetheless methodologies for the risk assessment of metals and organic compounds are described in the EC Technical Guidance Document of 2003 [73]. Similarly, veterinary medicines are covered by Directive 2004/28/EC [74] and Regulation (EC) No 726/2004 [75]. In 2007 the European Medicines Agency launched a guideline [76] on the assessment of veterinary medicines in support of two other guidance documents on the environmental risk assessment of veterinary pharmaceuticals adopted following the international harmonisation process through the Veterinary International Conference on Harmonisation [77; 78].

(Probabilistic) Risk Assessment

Ecological Risk Assessment is a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors [79]. This scientific step underpins the decision-making process defined as Risk Management – that involves considerations of political, social, economic, and technical factors – by providing information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard [80].

The standardization for terrestrial risk assessment has been addressed at the EU level [81] with an holistic approach selecting key route-receptor interactions for each assessment as mentioned by Tarazona *et al.* [82]. In a general way risk assessment methodology is based on the systematic and tiered comparison of the exposure (predicted environmental concentration – PEC) against the effects (predicted no effect concentration – PNEC) with the application of safety factors to account for uncertainty [66]. But as Calow [83] has pointed out already 15 years ago, when looking at the challenges for ecotoxicology in Europe “this is not quite risk assessment in the sense of explicitly characterizing the probability of populations or communities becoming impaired to defined extents”. A way to handle this bias is to include ecological considerations in risk assessment [84] or by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [85]. The last is called the probabilistic risk assessment approach where instead of point estimates a distribution for exposure (exposure/environmental concentration distribution – ECD) and/or effects (species sensitivity distribution – SSD), and concomitant risks may be obtained [86]. Probabilistic methodologies have been considered valid and scientifically sound or have been putted forward by many international bodies involved in the field of risk assessment, e.g., EC [66], SETAC [87], ECETOC [88], OECD [89] or USEPA [79]. And in an European Workshop on Probabilistic Risk Assessment for Pesticides [90], the main advantages of this approach were highlighted to aquatic organisms, and terrestrial plants, vertebrates and invertebrates: helps to quantify variability and uncertainty, can produce outputs with more ecological meaning (e.g. probability and magnitude of effects), makes better use of available data, identifies most significant factors contributing to risk, can provide an alternative to field testing or helps focus on key uncertainties for further study in the field, and promotes better science by considering multiple possibilities. Moreover when considering the probabilistic methods instead of the regular deterministic approach, risk assessment is more transparent, with the sources of uncertainty identified, allowing therefore a clearer communication of risk [81].

Risk Communication

Risk Communication is defined by the OECD [80] as the interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public. Thus communication is an important tool in the understanding of environmental problems, in the orientation of decision-making and ultimately inducing a cultural change towards sustainability [91]. But, although risk communication is recognized as part of the assessment of chemicals’ protocols in Europe [81; 66], in the USA [79], and at the international level, [80] it is not clear as it relates structurally to the assessment and the management phases. In the USA it is a differentiated step within risk analysis that includes risk assessment, risk management and risk communication [92], whereas in Europe it is included in the risk management process that comes after risk assessment [93]. Nonetheless the goal of risk communication is fully recognized, which is to enhance the likelihood that risk management decisions will incorporate the results of the risk assessment and that both the assessment and the decisions will be understood and accepted by potentially affected individuals or groups [94] as well as the general public. In spite of this, to our knowledge, there are not any available protocols or guidelines for communicating the risks of chemicals to the ecosystems, and most of risk communication processes are related to human health.

Objectives

The main objective of this dissertation was to assess and set the first steps for the communication of risks posed to the ecosystem from the chemical inputs due to extensive agriculture within a Mediterranean protected area.

It is perfectly clear the importance of halting the loss of biodiversity and to properly assess the utilization of chemicals in order to achieve a sustainable development in our society. But how far are these two subjects being brought together? How suitable are the available risk assessment protocols for protecting ecologically valuable areas? How is the reality towards the development and usage of tools for communicating risks?

If we look at the Streamlining European 2010 Biodiversity Indicators, amongst the 26 proposed indicators, only for indicator 19, nitrogen balance in Agriculture, environmental risk assessment is proposed as a tool for analysis of options. And when looking at the legislation for protection of biodiversity, the birds' directive or specific management plans like the Portuguese Sectorial Plan for the SPA of Castro Verde, the assessment of chemicals is disregarded albeit being mentioned the problems of the intensification of agriculture namely the increase of fertilizers and herbicides input.

Regarding European risk assessment protocols it may be observed that they are insufficient to protect ecological values in specific areas, because they only present generic models and exposure scenarios that do not cover different levels of biodiversity protection in different eco-regions. It would be important to re-evaluate chemicals being used in the protected areas, namely the Natura 2000 Network, and also to map the ecological interest of risk assessment at European level. After all what do we want to protect?

The importance of knowing the perception of people towards environmental risks that may affect biodiversity in order to develop tools for communication is fully recognized but seldom used in supporting the management of risks. Especially when dealing with ecosystems with conservationist concern it is essential to drive everyone's attention for the risks of activities independently of how little conspicuous the risks may be.

New tools for communication and risk assessment…

Bearing all the previous aspects in mind our work aimed to develop new tools for scientifically sound based environmental policy by:

a) Developing an innovative model with a specific exposure scenario in a Mediterranean area of concern in terms of biodiversity, allowing higher tier refinements based on biotic parameters for three bird species listed in annex I of the birds' directive, Great Bustard *Otis tarda*, Lesser Kestrel *Falco naumanni* and Montagu's Harrier *Circus pygargus*.
b) Using a probabilistic approach to characterize risks posed by the different chemicals that take part in the extensive agricultural activities in a Natura 2000 site, selecting examples in each group (Glyphosate as an herbicide, and Cadmium metal and LAS abundantly present in wastewater sludge) and using the frameworks proposed in the respective piece of legislation, guidance documents and/or risk assessment report;
c) Contributing for the development of a risk communication framework that takes into account the public awareness and perception of the risk, the necessity of illustrating the overall impression of the risk to farmers as the major actors in the continuation of extensive agricultural practices, and to make the risk assessment procedures part of risk management options to local authorities and decision-makers.

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CHAPTER 2

Chapter 2. A conceptual model for assessing risks in an European Mediterranean protected area

Abstract

Every year millions of tons of chemical products are disposed to the environment as a result of human activities, with deleterious consequences to biodiversity. In Europe the biodiversity policy basis for action is provided by the Birds and the Habitats Directives. According to these directives a network of protected areas is being built across EU countries encompassing the Natura 2000 Network. But the management plans of these protected areas do not require an ecotoxicological assessment of chemicals used within its limits. As for risk assessment protocols described in EC pieces of legislation and technical guidance documents, they are generic guidelines that not take into consideration regional particularities, e.g. the Mediterranean ecoregion specificities, and its local ecological values. Herewith we present a conceptual model for the assessment of risks posed by agriculture to bird species of conservationist concern from Natura 2000 Network sites; an example is set in a cereal steppe of the Iberian Peninsula. Hazards identified are related to the utilization of herbicides, disposal of sewage sludge to be used as fertilizer, and the input of veterinary pharmaceuticals that can be found in livestock dung and urine. This innovative model, to be used in high tier risk assessment, takes into account the biotic parameters of bird populations from this protected areas. The transfer of chemicals is considered to occur mainly through a realistic trophic chain scenario according to the different feeding behaviour among different species and even within the same species when having different feeding habits (e.g. adults and juveniles). Moreover, the probabilistic approach is proposed in order to perform a transparent risk assessment and clearer risk communication.

Keywords: conceptual model, probabilistic risk assessment, protected area, agriculture, herbicide, sewage sludge, veterinary medicinal product.

Introduction

According to the UN Programme of Action from the Earth Summit of Rio de Janeiro 1992, Agenda 21, improved risk assessment is necessary for the safe use of toxic chemicals (Section II, Chapter 19) [1]: "*Thousand of chemicals are used in every aspect of human endeavour but the long-term health and environmental risks of most of them are unknown. 95 % chemical manufacturing involves only 1500 chemicals but crucial data for risk assessment are lacking for many of them.*" In Europe the utilization of chemicals by human activities is regulated by several pieces of legislation implemented through guidance documents that foresee risk assessment protocols as the tool to set the impact of chemical contamination on biota [2]. With the introduction of REACH, Regulation (EC) No 1907/2006 [3], risk assessment processes for existing substances will be further regarded and hasten. But in spite of the many strides in reducing the chemical contamination and pollution its effects on human health and biodiversity are still quite evident. Maintaining the richness of European biodiversity and ecosystems is essential when considering present and future ecosystem services [4].

At the EU level a network of protected areas, Natura 2000 Network, is being built on the designation of areas for conservation under the EU Birds and Habitats directives. Endangered and rare birds at the European or global level were firstly addressed by the Birds Directive [5], but this piece of legislation was afterwards complemented by the Habitats Directive [6] where habitats and other wildlife species were also considered. Thus Member States have designated Special Protected Areas (SPAs) for wild birds and then proposed Sites of Community Interest (SCIs) for habitats and endangered species, that encompass the Natura 2000 Network. Once in Natura 2000 Network the conservation status of habitats and species listed in the nature directives must be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [5; 6].

This paper aims to present a critical review on protocols for the assessment of risks posed to Mediterranean protected areas, namely a bird SPA. Moreover herewith we present a generic approach with a site-specific conceptual model and exposure routes, that may take place due to the input of chemicals from extensive agriculture, for a cereal steppe in the Iberian Peninsula. The paper is structured as follows. A policy background is drawn in terms of the pieces of legislation and guidance documents that underpin the protection of nature and risk assessment protocols in Europe. A conceptual model and all its elements are described as well as the hypothetical exposure routes that may reach three bird species of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*), and Montagu's Harrier (*Circus pygargus*).

Policy Background

Natura 2000 Network

In the Birds Directive [5] it is mentioned that pollution as a result of man's activities may affect birds directly or may destroy their habitats. Whereas for the Habitats Directive [6] the need for Member States developing appropriate management plans with conservation measures is referred. Thus it would be expected that national management plans for Nature 2000 Network sites would foresee in depth all risks posed to the protected species and/or habitats such as chemical pollutants. As will be explained further on with two examples from the Iberian Peninsula this is not the case.

European Risk Assessment Protocols

In 2000 the EU's Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) published an opinion on the scientific basis for proper risk assessment on terrestrial ecosystems [7]. The driving force of this review was the fact that research activities regarding the environmental effects of pollution were dominated by the aquatic compartment and for terrestrial risk assessments the aquatic models had to be adapted. This fact had consequences at the regulatory arena and legislative initiatives considered terrestrial ecosystems of secondary importance or even disregarded it. A clear example lies in the fact that the Water Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy) came out in 2000 whereas the common protection of soils across the EU is yet to be implemented and a proposal for a Soil Framework Directive (COM(2006) 232) was only published in 2006.

Another breakthrough from the CSTEE report was the proposal for a more holistic hazard identification and conceptual model by selecting key route-receptor interactions for each assessment instead of the soil and above soil compartment approach [8]. Exposure scenarios should therefore include the feeding behaviour of species aiming to protect, contaminant concentrations in food as well as transfer of the chemical from soil to food. This way transfer of chemicals into the trophic chain is addressed and hence uptake by animals and plants by bioaccumulation and biomagnification are considered. Although a chemical may pose an acceptable risk for soil dwelling organisms it may represent an unacceptable risk for top predators due to biomagnification through the food chain.

But other important aspects of risk assessment of chemicals on terrestrial ecosystems were also addressed. As indicated by the CSTEE the assessment of effects is mainly aimed on the structure and function of the ecosystem guaranteeing therefore the human uses of the environment (e.g. soil used for agricultural purposes). Thus the protection goal is at population or community level. Nonetheless protected areas with special level of protection for highly endangered species may have to undergo specific risk assessment with the identification of effects at the individual level. Effects requiring an assessment at individual level, such as human health effects, require a different approach that was not considered by the CSTEE review. In fact risk assessment in areas of high ecological concern is still a bit cloudy in EU's protocols.

Problem Definition and Selection of Scenarios

The foundation of any ecological risk assessment is the clarification of the issue that is going to be evaluated – problem definition – as well as the hazards that will be covered by the evaluation and the respective sources – selection of scenarios [7]. Therefore herewith we will be presenting the environmental values to be protected and describe a model for a targeted, higher tier, risk assessment that includes biological receptors of conservationist concern and the exposure routes for chemicals that are hypothesized to pose risk to the community of birds from a Mediterranean protected area.

Case-study: a Mediterranean SPA

The highest number of plant and animal species in Europe is hosted in the Mediterranean basin, which has been identified by Conservation International as one of the world's 34 biodiversity hotspots [9]. The bimodal weather pattern, that provides the unity to this ecoregion, is dominated by hot, dry summers, and cool, wet winters, with average annual rainfall ranging from less than 100 millimetres in desert territories to more than 4000 millimetres on certain costal massifs. In the western Mediterranean, the Iberian Peninsula, for at least two months each year there is frequently no precipitation al all, and most plants and animals experience a water deficit thus having developed ecophysiological or behavioural adaptations [10].

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. Less than a fifth of the European land can be regarded as not directly managed. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [4]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [11]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems.

An important percentage of the Iberian Natura 2000 Network sites depend upon the continuation of extensive farming practices farmland – Portugal more than 25 %, Spain 18 %, EU-15 18 % –, that are extremely important refuges for several bird species. In December 2006, SPA for wild birds' sites covered 9.9 % of EU-25 territory but in the Iberian Peninsula this value was of 17 % [12]. Steppic areas are typical examples of HNV farmland from the Mediterranean region with bird assemblages of conservation concern [13]. In the Iberian Peninsula two of the most important sites for the conservation of bird species are, the SPA of Castro Verde (Alentejo, Southern Portugal) [14] and the SPA of the Cereal steppes of Jarama and Henares rivers (North of Madrid, Central Spain) [15]. In both SPAs, extensive agriculture of cereals in rotation with fallow land (normally used as pasture) create a steppic habitat perfect for the conservation of birds such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu's Harrier (*Circus pygargus*). But the traditional agriculture practices with low intensity non-irrigated cereal farming land represent a marginal economic system with a yield lower than the EU average and specially Atlantic Europe. Hence the main threats to these SPAs are the abandonment of the less fertile agricultural soils with the intensification of agriculture in the remaining land [16; 17].

Ecology of the Receptors

Since the 1970s it has been taking place an overall decline in farmland bird populations across Europe, a declining trend that is not apparent in bird assemblages of other habitats. This long-term trend suggests that the driver factors are specific to this habitat [18] and in fact agriculture intensification may account for the decline of more than 40 % of the bird species [13]. But as evidenced by Donald *et al.* (2006) [18] not all farmland species exhibited patterns of population decline. For the present risk assessment three species of birds were selected: the Great Bustard and the Lesser Kestrel that have a negative population trend of, respectively, -1.1 and -1.39; and the Montagu's Harrier that exhibits a positive mean population trend of 0.64. Thus a typical gregarious omnivorous bird (Great Bustard) and two predators (Lesser Kestrel and Montagu's Harrier) were chosen allowing a different approach in the assessment because the predators, having a larger foraging area obtain food from a relatively larger area with different levels of contamination, and being in the top of the food chain may be exposed to a higher level of contamination due to biomagnification of chemicals.

*Great Bustard (*Otis tarda*)*

The Great Bustard (*Otis tarda*), family *Otididae*, is one of the largest birds of Europe and one of the heaviest flying birds of the world, typical of the steppic habitats and open lands with non-intensive farming [19]. Its populations though widely distributed, from the Iberian Peninsula to eastern Asia, are generally separated and consist of a few tens to several hundred individuals [20]. *O. tarda* is listed in the annex I of the Birds Directive [5] as well as categorized as VULNERABLE in the 2007 IUCN Red List [21].

This species has an accentuated sexual dimorphism with males weighting around 16 kg, which is 2 to 4 times the weigh of females (3-4 kg) [22], and measuring up to 1 m being therefore ca 50 % bigger than females [21]. The Great Bustard is a gregarious bird that lives in flocks with a variable number (4-16) of individuals around the year [22].

Feeding is the most time-consuming activity for the *O. tarda* [23]. They are omnivorous birds that feed mainly upon green plant material, and arthropods and seeds to a lesser extent [19; 20]. In fact green plant material accounts for 71 % in summer and autumn, and 95 % in spring and winter [24]. As for juveniles, fed by rearing females, the diet is based on invertebrates [20].

*Lesser Kestrel (*Falco naumanni*)*

The migratory Lesser Kestrel (*Falco naumanni*), Family *Falconidae*, has a Mediterranean range for breeding, heading south to Africa in winter, particularly to the southern Sahara region. It forages steppic habitats and grasslands with non-intensive cultivation [25]. *F. naumanni* is listed in the annex I of the Birds Directive [5] as well as categorized as VULNERABLE in the 2007 IUCN Red List [25].

This small hawk measures ca 30 cm and seldom exceeds 200 g of weight. Its rusty plumage bears him the right camouflage for the arid habitats where it lives [26; 25]. The Lesser Kestrel is a gregarious raptor that feeds mainly upon insects and preying activity tends occur in the surroundings of the colonies [27].

*Montagu's Harrier (*Circus pygargus*)*

The Montagu's Harrier, Family *Accipitridae*, has a widespread but patchy breeding distribution in Europe, which constitutes over 50% of its global breeding range [28], and it winters sub-tropical Africa and India, and around the Mediterranean Sea [29]. Although it is originally a marsh harrier it colonized the great extent of farmland that covers Europe [30]. *C. pygargus* is listed in the annex I of the Birds Directive [5], and considered of LEAST CONCERN in the 2007 IUCN Red List [28].

Being the smallest within the Harriers, this species measures 43-47 cm [29] and weights around 345 g although males tend to increase in body size as one goes west and south in Europe [30]. It is frequent to observe the reunion of couples in colonies while breeding showing therefore gregarious behaviour [31]. The Montagu's Harrier diet includes small mammals, mainly rodents, and occasionally small birds and large insects [32].

Hazard Identification

Both the management plans from the Portuguese [14] and the Spanish [15] identify the intensification of agriculture as a major threat to the conservation of the birds from the referred SPAs. But due to the present agriculture system chemicals are permitted to use despite the fact that its ecotoxicological evaluation is not foreseen. The exemptions are the prohibition of some pesticides (herbicides and fungicides) in the case of agri-environmental financing under Common Agriculture Policy (CAP) and all chemicals in certain areas as a result of compensation measures following impact assessments of road infrastructures.

This is the case of herbicides applied to the soil before seedling, according to the agronomic application rate, in order to control the competing vegetation. Herbicides are extensively used in agriculture but are negatively correlated with biodiversity [4], namely plants, invertebrates and birds [33].

The existence of livestock grazing in the area creates a less conspicuous pathway for the input of high local concentrations of toxicants into the ecosystem due to the veterinary pharmaceuticals that can be found in dung and urine [34]. The extensive livestock farming is most certainly a vector for this type of contamination. Veterinary medicines are important safeguarding health and welfare of livestock [35] but may have a potential impact in terrestrial ecosystems [36].

Domestic Sewage Sludge is also applied as fertilizer and soil amendment and at the same time as way for the disposal of waste sludge [37]. Previous studies on the use of sewage sludge as fertilizer to soil under Mediterranean climatic conditions revealed an impoverishment of the community structure and decrease in the diversity of microarthropod populations [38; 39]. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [40]. As for the nutrients present in the sludge they are considered to produce negligible risk for the soil compartment [7].

The Conceptual Model

Herewith we present a conceptual model for refining local risk assessments in sites with specific ecological values (figure 2.1). This innovative model is to be used in a high tier risk assessment by taking into account the biotic parameters of bird population from protected areas. The transfer of chemicals is considered to occur mainly through a realistic trophic chain scenario and that is why the different feeding behaviour is considered among different species and even within the same species when having different feeding habits (e.g. adults and juveniles).

According to the management plans of the SPA, the crop production should take into consideration the supply of food for the populations of wild birds. Therefore when birds graze the farmlands or prey upon invertebrates and small mammals that inhabit those fields, are expected to uptake the chemicals that are disposed into soil, through the food chain.

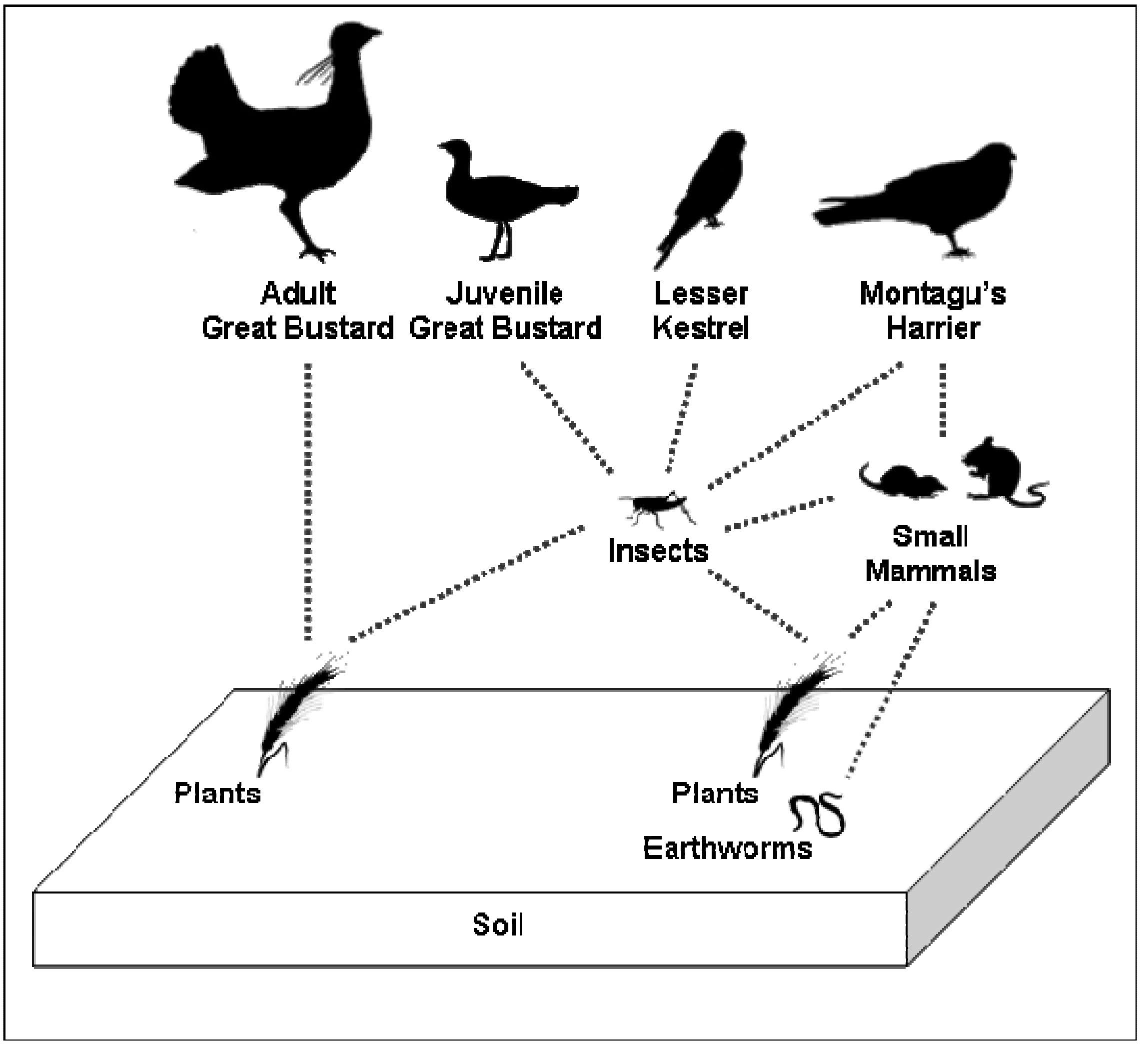

Figure 2.1. Conceptual model for toxicant pathways to the ecological receptors of a food chain from an extensive agriculture habitat.

Before seedling, herbicide for weeds is sprayed in the agriculture fields. In some areas of the farmland, sewage sludge is incorporated into soil. In the areas where grazing takes place, veterinary products are expected to accumulate in soil. Plants and soil-dwelling animals (e.g. earthworms) may bioaccumulate chemicals from soil pore-water [7]. Herbivorous animals – adult Great Bustards, insects and small mammals – may then uptake the chemicals from plants. From insects, the chemicals may pass to the animals that prey them – juvenile Great Bustards (fed by rearing females), Lesser Kestrels, small mammals and Montagu's Harriers. The top predator of the food chain, the Montagu's Harrier, may also uptake the chemicals from preying upon from small mammals that in turn may have been feeding on plants and invertebrates.

Chemicals Assessment

Besides a proper conceptual model, CSTEE [7] also suggests the refinement at exposure and effects assessment steps. Instead of the default values from worst case scenarios real emission data should be used and presented in the form of probability functions for the predicted environmental concentrations. For studying the effects a combined approach of bioaccumulation tests in terrestrial microcosms [41; 42] may be performed in order to attain information on the interactions between different trophic levels, as well as on the transfer of chemicals through the trophic chain.

Risk assessment in protected areas should be used for regulatory purposes in order to safeguard its ecological values as defined by the directives that underpin the Nature 2000 Network. Hence the assessment of chemicals in those sites must be developed in accordance to the generic protocols described in the EU pieces of legislation and guidance documents. In the Annex VI of the Directive 91/414/EEC the detailed evaluation and decision making criteria for plant protection products (e.g. herbicides) is described [43]. Additional technical guidance is presented in the Guidance Documents on Terrestrial Ecotoxicology [44] and Birds and Mammals [45] in support of the directive and in the outputs of the recent European Food Safety Authority scientific workshop on the revision of a guidance document on assessment of pesticide risks for birds and mammals [46]. Data for the risk assessment of sewage sludge may be found in the EC Directive 86/278/EEC on the use of sewage sludge in agriculture [47], namely the limit values for heavy metals, but limits for organic compounds are not included. The limit values for organic compounds can only be found in the EC Working Document on Sludge [48] on the revision of the Directive 86/278/EEC. Nonetheless methodologies for the risk assessment of metals and organic compounds are described in the EC Technical Guidance Document of 2003 [49]. Similarly, veterinary medicines are covered by Directive 2004/28/EC [50] and Regulation (EC) No 726/2004 [51]. In 2007 the European Medicines Agency launched a guideline [52] on the assessment of veterinary medicines in support of two other guidance documents on the environmental risk assessment of veterinary pharmaceuticals adopted following the international harmonisation process through the Veterinary International Conference on Harmonisation [53; 54].

Probabilistic Risk Assessment

In a general way risk assessment methodology is based on the systematic and tiered comparison of the exposure (predicted environmental concentration – PEC) against the effects (predicted no effect concentration – PNEC) with the application of safety factors to account for uncertainty [2]. But as Calow [55] has pointed out already 15 years ago, when looking at the challenges for ecotoxicology in Europe "this is not quite risk assessment in the sense of explicitly characterizing the probability of populations or communities becoming impaired to defined extents". A way to handle this bias is to include ecological considerations in risk assessment [56] or by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [57]. The last is called the probabilistic risk assessment approach where instead of point estimates a distribution for exposure (exposure/environmental concentration distribution) and/or effects (species sensitivity distribution), and concomitant risks may be obtained [58]. Probabilistic methodologies have been considered valid and scientifically sound or have been putted forward by many international bodies involved in the field of risk assessment, e.g., EC [2], SETAC [59], ECETOC [60], OECD [61] or USEPA [62]. And in an European Workshop on Probabilistic Risk Assessment for Pesticides [63], the main advantages of this approach were highlighted to aquatic organisms, and terrestrial plants, vertebrates and invertebrates: helps to quantify variability and uncertainty, can produce outputs with more ecological meaning (e.g. probability and magnitude of effects), makes better use of available data, identifies most significant factors contributing to risk, can provide an alternative to field testing or helps focus on key uncertainties for further study in the field, and promotes better science by considering multiple possibilities. Moreover when considering the probabilistic methods instead of the regular deterministic approach, risk assessment is more transparent, with the sources of uncertainty identified, allowing therefore a clearer communication of risk [7]. Thus another refinement to the assessment of the present conceptual model may be the utilization of the probabilistic approach.

Final Remarks

The nature conservation instruments across Europe that settle the Nature 2000 Network and respective management plans in each Member State completely disregard ecotoxicological tools for setting the impact of pollution on biota. On the other hand, risk assessment methodologies from EU legislation and respective guidance documents are generic protocols that may not protect the ecological values from areas of conservationist concern. For instance the adaptation of the methodologies and basic principles of the EC Technical Guidance Document on Risk Assessment [49] to the biology and ecology of the species that represent the ecological values will allow a suitable assessment of chemicals in designated areas. Furthermore has shown by Faber [64], with a site-specific approach for risk assessment, by differentiating the level of protection for the chosen effect criteria depending on land use, a greater relevance of results will be achieved.

Therefore the development of innovative conceptual models with realistic exposure scenarios may be used to assess the risk of chemicals in protected areas such as SPAs for wild birds. Moreover there is a need for refinement of targeted assessments by taking into consideration the specificities of the Mediterranean ecoregion, and addressing real emission data with the occurrence of biomagnification through the foodchain where protected bird species are ecological receptors. Hence probabilistic methodologies have been putted forwarded as a way to perform transparent risk assessment, clearer risk communication, use of all available data, and to identify the main sources of uncertainty [7].

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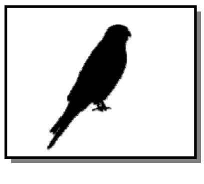
CHAPTER 3



Chapter 3. Assessing the risk of cadmium to birds from sludge applications in an European Mediterranean protected area

Abstract

While supplying some essential plant nutrients and enhancing soil organic matter, sewage sludge may be responsible for the input of heavy metals to the terrestrial compartment. Using Cadmium as a model metal, potential avian risks within a bird special protection area (SPA) of the European Natura 2000 Network were assessed. The selected case-study was the SPA of Castro Verde, Southern Portugal (Alentejo), the most important Portuguese area for the conservation of steppic bird species. Terrestrial microcosms were used for studying the bioaccumulation along the food chain of bird species of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). A deterministic scenario and four probabilistic scenarios with increasing Cd concentrations were assessed. Main differences among risks posed to birds are due to diet and concomitant pathways of Cd through the food web. The most critical food chain contribution is that related to the exposure of the top predator Montagu's Harrier, followed by those associated to the exposure of the juvenile Great Bustard, the Lesser Kestrel and the adult Great Bustard, respectively.

Keywords: probabilistic risk assessment, protected area, agriculture, sewage sludge, cadmium, *Otis tarda*, *Falco naumanni*, *Circus pygargus*.

Introduction

The special protection area (SPA) of Castro Verde in southern Portugal, Alentejo, is classified under EU's Nature 2000 Network for the conservation of endangered birds in accordance to the Council Directive 79/409/EEC (birds' directive). According to the Portuguese Institute for Nature Conservation [1] this SPA is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*), the Lesser Kestrel (*Falco naumanni*), or the Montagu's Harrier (*Circus pygargus*). The SPA of Castro Verde includes six municipalities of which the municipality of Castro Verde has the higher percentage of land, 55 %, out of a total area of 79007.17 ha. In the last forty years average annual temperature from this region was approximately 22 ºC and annual rainfall 500-600 mm. For the same time span, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [2]. The main landscape is characterized by a mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [3; 4].

In recent years in Castro Verde, wastewater sludge was used as fertilizer in a program aiming to prevent desertification and soil erosion [5] but these products were not assessed for the risks to the ecosystem in spite of the performed chemical analysis. On one hand sewage sludge supplies some essential plant nutrients and impart soil property enhancing organic matter, on the other hand it holds a complex pollutant burden of organic pollutants and heavy metals [6]. The presence of metals in sludge is a clear concern and is regulated by the Sewage Sludge Directive 86/278/EEC [7]. Cadmium can be used as a model metal. According to the risk assessment report (RAR) for Cadmium (Cd) edited by the European Chemicals Bureau [8] sewage sludge is a minor source of Cd for soils on an average basis; but it is a major source of Cd in soils where sludge is applied.

Terrestrial micro and mesocosms have been pointed out as important tools for higher tier risk assessments by gaining insight on the interactions between different trophic levels, as well as on the transfer of chemicals through the trophic chain [9]. Some ecosystem surrogate methodologies have been developed and presented. Terrestrial Model Ecosystems (TME) consist of enclosed intact soil-cores containing biota (plants, animals and microbes) from selected field sites [10] whereas in the Multi-Species Soil Systems (MS 3) columns of natural sieved and homogenised soil are used being the organisms (plants and invertebrates) deployed afterwards [11]. Further refinement on risk assessment may be performed using probabilistic methodologies by applying numeric factors that increase the exposure/effects estimate with a Monte Carlo simulation [12]. Thus instead of point estimates a distribution for exposure (exposure/environmental concentration distribution) and/or effects (species sensitivity distribution), and concomitant risks may be obtained [13].

This paper evaluates the hypothesis that Cd present in sewage sludge may undergo bioconcentration through the food chain and pose risk to some bird species of conservationist concern from the SPA of Castro Verde. For the purpose a conceptual model previously presented [CHAPTER 2] was used to assess, with increasing refinement, the risks of Cd metal. Terrestrial microcosms were used for studying the uptake from the different organisms of the food chain reaching the considered ecological receptors: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*).

Methodology

Soil Characterization

Agriculture soil collected from the top 10 cm layer in a field from the SPA of Castro Verde was sieved *in situ* with a 4 mm mesh. A site at the "Herdade de Vale Gonçalinho" (N 43º 14'21 6'', W 8º 30'35 3'') was chosen that had not received manure, sewage sludge or pesticide applications during the last decade. When brought to the laboratory, the soil was left at dark and aerated conditions for one week. The soil was characterized for basic pedological descriptors such as coarse sand (23.3 %), fine sand (37.0 %), silt (24.9 %), and clay (14.8 %); and physical-chemical properties: pH (6,1), residual humidity (4 %), density (1.21), maximum water holding capacity (27.55 %), NH_4^+ content (1 ppm), oxidizable C (1.74 %), total organic matter (3 %), extractable P (60 ppm) and extractable K (98 ppm), in accordance to the methodology proposed by the British Society of Soil Science [14].

Experimental Design

Test species

Three species of birds included in annex I of the Birds Directive [15] were chosen as model ecological receptors: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). While the first two have had a decreasing trend in Europe the last has shown a slight increase in recent years [16]. A conceptual model for the assessment of risks to these birds was presented elsewhere [Chapter 2]. In short the model is based in the diet of each species and the respective food chain (figure 3.1). Adult Great Bustards feed on plants and juveniles eat the insects rearing females provide them. The raptors, Lesser Kestrel and Montagu's Harrier, prey upon insects, and insects and small mammals (herbivorous and insectivorous), respectively. Hence *C. pygargus* may be exposed to higher levels of contamination due to bioaccumulation of chemicals through the food chain. Moreover, birds of prey have larger foraging areas and may obtain food from places with different levels of contamination.

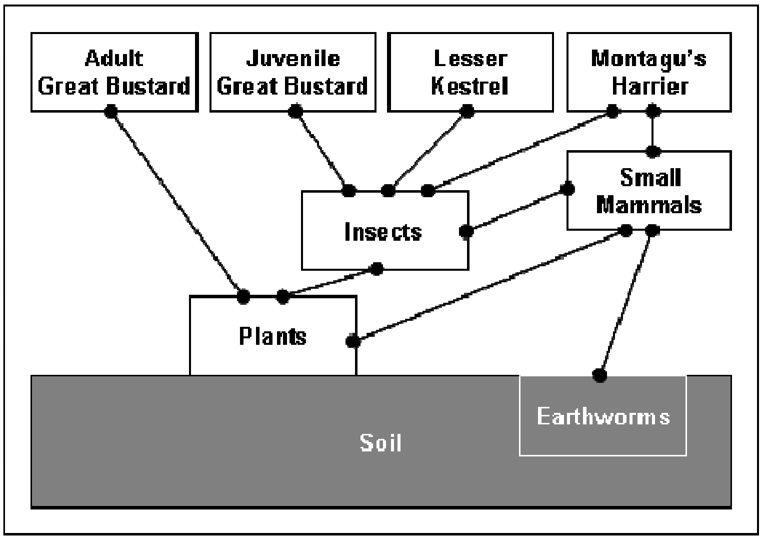


Figure 3.1. Food chain of the selected ecological receptors (protected bird species) from the SPA of Castro Verde.

Laboratory tests were performed with plants and invertebrates, as key elements of the food web, in order to determine the bioaccumulation factors (BAF) for each taxonomic group. Plant species existing in the SPA of Castro Verde were chosen in accordance to *O. tarda* feeding preferences: common wheat (*Triticum aestivum*), chickpea (*Cicer* *arietinum*), and radish (*Raphanus* sp.) [17-19]. But these plant species are also important in the local agricultural scheme since *T. aestivum* is sown in the first year of the rotation system as the primary cereal (in the second year the secondary cereal is sown, oat *Avena sativa*, following two or three years of fallow depending on soil fertility) [1]. Cereals are usually sown in September-November and harvested in June-July [20] and leguminous crops (*C. arietinum*) are sown in smaller amounts in summer. A typical Mediterranean weed *Raphanus raphanistrum* was also intended to be tested but since no commercial seeds of wild radish were available cultivated radish (*Raphanus sativus*) was used instead. Earthworms *Eisenia andrei*, cultured in our lab, were tested as key elements involved in secondary poisoning of organisms that feed upon them [8], and due to the role they play in water infiltration and storage and soil aeration [21] thus contributing for the mobilization of metals [22]. Insects from the order Orthoptera are important food items for juvenile *O. tarda* [18] but also to *F. naumanni* [23] and to a less extent to *C. pygargus* [24]. The locust *Schistocerca gregaria*, late first or early second instars, were acquired from Blades Biological Ltd (http://www.blades-bio.co.uk/) and left to acclimate to laboratory testing conditions during one week while fed *ad libitum* with dry bran and fresh grass.

Terrestrial microcosms

For the experiments with terrestrial microcosms, equipment from the terrestrial model ecosystem (TME), field validated and ring-tested in an EU project [10] ("The use of TME to assess environmental risks in ecosystems", Project No: ENV4-CT97-0470), was used. Soil is contained in 40-cm long high-density polyethylene tubes (17.5 cm diameter) with a plate of the same material at the bottom, and a thin inert gauze to fit between the drilled holes of the bottom plate. The TME tubes are placed into moveable carts; in each cart up to 18 cylinders may be placed and the temperature inside may be controlled with a cooling unit in order to adjust soil temperature. Rain-heads made out of plexiglass (16.5 high and 14 cm diameter, with 12 evenly spaced holes where micro-pipettes are inserted) may be positioned above TME tubes for watering the soil and simulate rainfall.

Originally this equipment is used with intact soil cores, extracted from field without disturbing soil organisms and layers, being the encased soil-cores defined as TME [25]. But instead, for the present experimental work, soil was sieved as proposed by Fernandez *et al.* [11] for the Multi-Species Soil Systems (MS·3). Thus according to the SETAC workshop on “Semi-field methods for the environmental risk assessment of pesticides in soil” [26] sieving draws the line between microcosms and mesocoms and therefore in the methodology presently addressed the term TME cannot be used. This methodological alternative offers a compromise between cost, realism and reproducibility [22]. While overcoming the high variability of mesocosms [27] it reasonably surrogates agricultural (arable) soils where structure and biota are modified [11; 22].

Experimental set-up

Laboratory conditions were adjusted to simulate environmental conditions from spring and autumn in order to address climatic circumstances when, respectively, chickpea and common wheat are sown: photoperiod with light/dark cycles of 10/14 h (8000 lux light intensity, provided by Philips SON-T Agro high pressure sodium lamps); soil temperature 12 ± 2 ºC; laboratory temperature 20 ± 2 ºC; air moisture inferior to 40 %; and rainfall simulation with 150 ml, two times per week, in each TME (total 1.2 l simulating monthly ca 55 mm rainfall). Under Mediterranean conditions a great resemblance exists in weather patterns of spring and autumn.

The experiment was carried out for 28 days with the following scheme:

- At day -1, soil columns were prepared with 10 kg of sieved soil saturated with a volume of 1.3 l of distilled water to get ca 70% of the soil water holding capacity. Before soil was placed into the treatment TMEs it was contaminated by dissolving Cd (cadmium chloride, CAS No: 25155-30-0) in distilled water that was then mixed with soil.
- Seeds (15 chickpea, 20 common wheat and 20 radish) were sown as proposed by OECD guidelines [28; 29] and 10 pre-weighted adult earthworms (with clitellum) were deployed as described in an international ring test for Cd bioaccumulation [30], per column at day 0. Six columns were allocated to the control and other six were allocated as treatment columns per cart. Four carts were used giving a total of 24 control and 24 treatment replicates.
- At day 14 three locusts were deployed per column; in one column per control and treatment in each cart, no locusts were deployed in order to evaluate plant growth and metal concentration at the end of the experiment.

- In the end of the experiment, day 28, aerial part of the plants was cut off and weighed; locusts were collected for survival assessment and the ones that survived were weighted and analysed for the presence of Cd. The soil columns were removed from the cylinder and homogenised in order to take samples that were analyzed for humidity and Cd. Earthworms were collected for survival assessment and the ones that survived were weighted and analysed for the presence of Cd.

Cd Analysis

For the analysis of Cd, soil, plants, invertebrates and leachate samples were digested with HNO_3 suprapur by means of microwave-assisted extraction following the US EPA evaluation methods [31-33]. Cd concentrations were then determined with Atomic Absorption Spectometry (Graphite Furnace AAS; Perkin Elmer Model Analyst 800).

Risk Assessment – scenarios and probabilistic assumptions

The chemical analysis of the wastewater sludge used in Castro Verde indicated a Cd concentration of 3.3 mg $Kg^{-1}_{Sludge\ dw}$ [5]. Firstly a worst case scenario (scenario 1) was assessed by considering a concentration of Cd in soil of the same order of magnitude from sludge; thus a concentration of 5 mg $Kg^{-1}_{Soil\ dw}$ (4 mg $Kg^{-1}_{Soil\ ww}$) was tested in microcosms allowing to determine environmental concentrations in each compartment and the bioaccumulation factors for plants and invertebrates. Afterwards the refinement of exposure assessment (Predicted Environmental Concentration, PEC) with the probabilistic approach was developed by:

- Using a realistic scenario for the exposure of Cd (scenario 2). According to the managers of the project of desertification prevention in Castro Verde [5], a range of 32-40 m^3 ha^{-1} of sewage sludge was amended to soil which meant the amendment of 5-6 T ha^{-1} at depth of 30-75 cm, in sites with total area of 2 ha. The value of Cd estimated to be deployed per ha was of ca 4 g, which represents a concentration of 0.5 µg $Kg^{-1}_{Soil\ ww}$.
- Considering the maximum level of Cd permitted by the Sewage Sludge Directive 86/278/EEC [7] (and national legislation, n.º 118/2006 [34]) to be added to

agricultural land (scenario 3), which is of 150 g ha^{-1} per year, that corresponds to a concentration of 19.6 µg $Kg^{-1}_{Soil\ ww}$.

- Modelling the realistic scenario and adding the concentration of Cd from the PEC for a generic Regional environment (PECregional) calculated in the RAR [8] based on the mass balance of Cd including detailed Cd immision onto soil from atmospheric deposition (scenario 4). Thus adding 48 µg Kg^{-1}_{Soil} to the realistic scenario means a final concentration of 48.5 µg $Kg^{-1}_{Soil\ ww}$.
- Covering temporal variation (scenario 5) from the experimentally obtained data by including increasing PEC values over time in plants and invertebrates due to accumulation. As an assumption Cd was considered to be continuously uptaken over time.

BAF values for plants and invertebrates were obtained from a concentration of Cd in soil of the worst case scenario, i.e. 4 mg $Kg^{-1}_{Soil\ ww}$. For the refinement scenarios BAFs were assumed to be the same as in the higher soil Cd concentration.

The formulas used for the calculation of exposure assessment were adapted from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [35]. BAF for plants and invertebrates were obtained: from the organism/soil Cd concentration ratio for plants and earthworms, and from locusts/plants ratio in the case of *S. gregaria* observed in our experimental study. Considering the limited exposure duration, the highest values were employed as representative. For small mammals, the accumulation of Cd increases with time, thus whole body BAF values were calculated with the following formula, $BAF = \alpha * F * ((1 - k_2) * Lifespan)$, where α is the fraction of ingested dose that is absorbed, F is the food intake rate per body weight (calculations are presented in the annex 3.1, table A.3.I), and k_2 is the rate constant for depuration; α and k_2 were obtained from the values presented for mammals in the RAR for Cd, being the k_2 corrected by the *Lifespan*, the maximum period of time that mammals can accumulate Cd [8]. PEC calculations for the ecological receptors are given by the formula: $PEC_{Bird} = (FIR/bw) * C * PD$, where C is the concentration of Cd in fresh diet and PD is the fraction of food type in diet [36].

For the assessment of effects – Predicted No Effect Concentrations (PNEC's) – of the target birds, the value suggested from the Cd RAR [8] was used following the $PNEC_{oral}$ for birds due to secondary poisoning, i.e. 0.16 mg kg^{-1}_{ww}. When characterizing the risk, a safety of factor of 10 was applied for covering the variability within bird species following the principles of the Technical Guidance Document on Risk Assessment [37] and considering that as the assessment is based on doses instead concentrations, the additional factor of 3 for covering lab to field differences in the food energetic content is not required here.

Probabilistic assessment was developed with Crystal Ball software [38] for Monte Carlo Analysis with 10000 trials. In scenarios 2 to 4, BAF values and respective standard deviations for plants and invertebrates calculated from concentrations obtained in the microcosms experiments were set as assumptions with normal distribution (figure 3.2) (annex 3.1, figure A.3.1). Also in scenarios 2 to 4, BAF for small mammals are dependent of the age of its populations; hence a triangular distribution was defined from weaning to maximum lifespan with the likeliest age of two thirds of the lifespan (figure 3.2) (annex 3.1, figure A.3.2) (age values obtained from Blanco *et al.* [39]). In the fifth scenario the temporal scale was considered with consequent increase in Cd uptake; this was modelled by considering a linear distribution assumption in plants and invertebrates, where the lowest value is the baseline concentration (given by control concentrations) and the highest value corresponds to concentration measured in treatment in the last day of the experiment (figure 3.2) (annex 3.1, figure A.3.3).

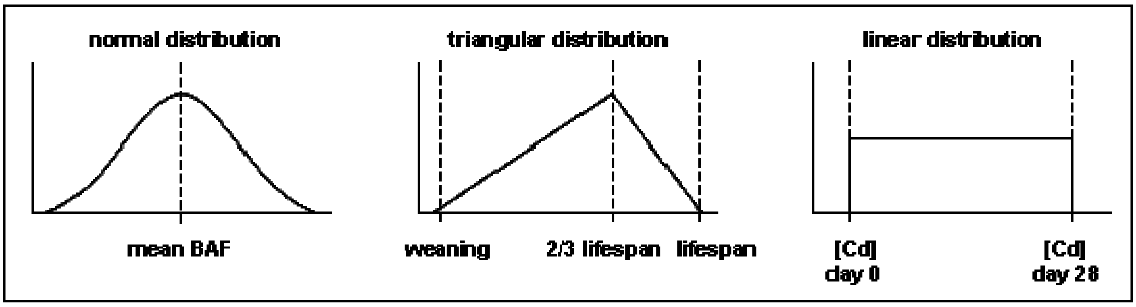


Figure 3.2. Probabilistic assumptions defined for the Monte Carlo Analysis of the scenarios.

Statistics

The comparisons between microcosms in terms of biological parameters, were analysed using one-way ANOVA with the SigmaStat statistical package [40]. Statistical analysis was carried out with a significance level of $p < 0.05$.

Results

Bioaccumulation

In order to determine Cd concentration a minimum quantity of material was needed – 2 g of soil, 150 mg of earthworm, 200 g of plant and 150 g of locust – what limited the usage of microsoms from carts in separate. Since the quantity of soil and *E. andrei* biomass was enough, Cd concentration was analysed in all carts. Plants and *S. gregaria* were only analysed for Cd in two carts.

Cadmium concentration in control soil (table 3.I) is quite low (0.057 mg kg^{-1}_{dw}) when compared with baseline concentrations for agricultural soils from another Mediterranean region (0.7 mg kg^{-1}_{dw}) [41], but exactly matches the background concentrations presented in the Cd RAR at the regional level (0.048 mg kg^{-1}_{ww}) [8]. The highest concentrations of Cd in control organisms are present in soil dwelling *E. andrei* where metal was analysed after guts were voided.

Table 3.I. Cd concentrations (wet weight) in soil and in the organisms from control and treatment (± standard deviation) microcosms, and bioaccumulation factors (wet weight) for plants and invertebrates.

Cd Concentration (mg kg^{-1}_{ww}) in Control Microcosms					
Soil	***E. andrei***	**Plants**			***S. gregaria***
		C. arietinum	***T. aestivum***	***R. sativus***	
0.048	0.458	0.003	0.039	0.041	0.099

Cd Concentration ± SD (mg kg^{-1}_{ww}) in Treatment Microcosms					
Soil	***E. andrei***				
4.18 ± 0.36	17.16 ± 2.88				
Soil		**Plants**			
		C. arietinum	***T. aestivum***	***R. sativus***	
4.21 ± 0.30		0.71 ± 0.38	5.66 ± 2.01	4.14 ± 2.36	
		Plants			***S. gregaria***
		C. arietinum	***T. aestivum***	***R. sativus***	
		0.64 ± 0.32	5.08 ± 1.42	4.18 ± 1.64	17.21 ± 5.36

BAF ± SD (kg_{ww} kg^{-1}_{ww})					
	E. andrei	**Plants**			***S. gregaria****
		C. arietinum	***T. aestivum***	***R. sativus***	
	4.13 ± 0.77	0.17 ± 0.10	1.36 ± 0.52	0.98 ± 0.50	1.67 ± 0.08

*** BAF for locusts was calculated taking into consideration its feeding preferences, 63 % *Cicer*, 14 % *Triticum* and 23 % *Raphanus*.**

BAF values are higher in *E. andrei* (table 3.I) than in the other tested invertebrate *S. gregaria*, which is in accordance with the trend of bioaccumulation factors reviewed in the RAR for earthworms and arthropods [8]; Cd is also concentrated (BAF > 1) in wheat. In previous studies on the bioaccumulation of Cd through the food chain [42; 43] its bioconcentration from soil to *T. aestivum* and to wheat phloem-feeding feeding aphids was also demonstrated. In some microcosms no locusts were deployed, giving us and average plant biomass growth. The difference between the average growths in microcosms with locusts allowed the inference of the feeding preferences of *S. gregaria* that had to be taken into account for the BAF calculation because the different tested plant species bioaccumulate different concentrations of Cd.

Deterministic BAF values for small mammals are as follows: herbivorous, weaning 0.2 kg kg^{-1}, two thirds of lifespan 2.1 kg kg^{-1} and lifespan 3.1 kg kg^{-1}; insectivorous, weaning 0.6 kg kg^{-1}, two thirds of lifespan 6.6 kg kg^{-1} and lifespan 9.9 kg kg^{-1}.

The EU RAR [8] and other ecotoxicological studies on contaminated land (e.g. [44-48]) offer a review of the accumulation pattern of Cd and basically conclude that in general, the concentration in the exposed organisms increases with the exposure time and experimental conditions and age in field studies, suggesting the steady state is not, at least rapidly achieved. In addition, the bioaccumulation factors tend to decrease with the increase in the soil cadmium concentration. As a consequence, the selection of the BAFs represents a critical element for the risk assessment. Two complementary approaches have been used in this study. First, the maximum BAF obtained in the experimental study for each species; second, an alternative to the BAF approach, using actual measured concentrations including the temporal variability. Both approaches were selected after considering the exposure route assessed in this study, and are expected to offer worst-case potential exposure conditions. The experimental BAFs and the increases in concentrations after Cd application were obtained for a relatively high concentration of Cd to the soil, equivalent to that expected in the sludge. This approach represents the worst case situation, unrealistic if an homogeneous distribution is assumed, but potentially realistic during a limited time period for those areas with no or very limited mixture of sludge within the soil, considering also the potential for attraction of soil dwelling animals (particularly relevant as the soil organic matter content is very low) and for significant plant growth (as the sludge offers a huge amount of nutrients). The exposure duration was sufficiently short for avoiding the inverse relationship between concentration and BAF, as demonstrated through the comparison with the BAF observed for the control samples. Hence, the selected approach can be considered appropriate for estimating the maximum potential Cd concentration in biota at the very local level related to sludge applications.

Exposure

PEC results for birds (figures 3.3 e 3.4) are given as point estimates from the deterministic approach – scenario 1 – or exposure distribution of probable occurrences within a defined range of Cd concentration, depending on the scenario, resulting from the Monte Carlo analysis (probabilistic approach) – scenarios 2 to 5. Already in scenario 1 it can be observed that following our hypothesis, at least for Montagu's Harrier, a biomagnification of Cd takes place from soil (4.2 mg kg^{-1}) through the food chain to the top predator (6.36 mg kg^{-1}). In view of the diet described for *C. pygargus* by Corbacho *et al.* [24], the following percentages of food were considered for the PEC calculations: 70 % herbivorous small mammals, 20 % insectivorous small mammals, and 10 % locusts. The refinement of exposure assessment with probabilistic analysis and the increase in Cd soil concentration from scenario 2 to 5 stresses the deterministic results and higher concentrations are present in Montagu's Harrier than in other bird species. As a result of the normal distribution assumptions for BAF in plants and invertebrates pathways for Cd uptake, the frequency of accumulation in birds from scenarios 2 to 4 also follows a normal distribution pattern. In scenario 5 it was assumed that the accumulation of Cd in plants and invertebrates does not always corresponds to the maximum measured value, but can be any concentration within the range measured in the experimental study. The rationale is that the consumer may feed randomly on plants/invertebrates of different ages, and therefore different accumulation levels. This leads to a pseudo-linear distribution of the Cd distribution in birds depending on the soil-plant-locust pathway, i.e. juvenile Great Bustard and Lesser Kestrel. The PEC from adult Great Bustard results only from ingestion of plants and was calculated by considering the different percentage of families present in the diet described by Palacios *et al.* [17] and represented in the present microcosm experiment, Fabaceae (40 %) for *C. arietinum*, Brassicaceae (32 %) for *R. sativus*, and Poaceae (28 %) for *T. aestivum*.

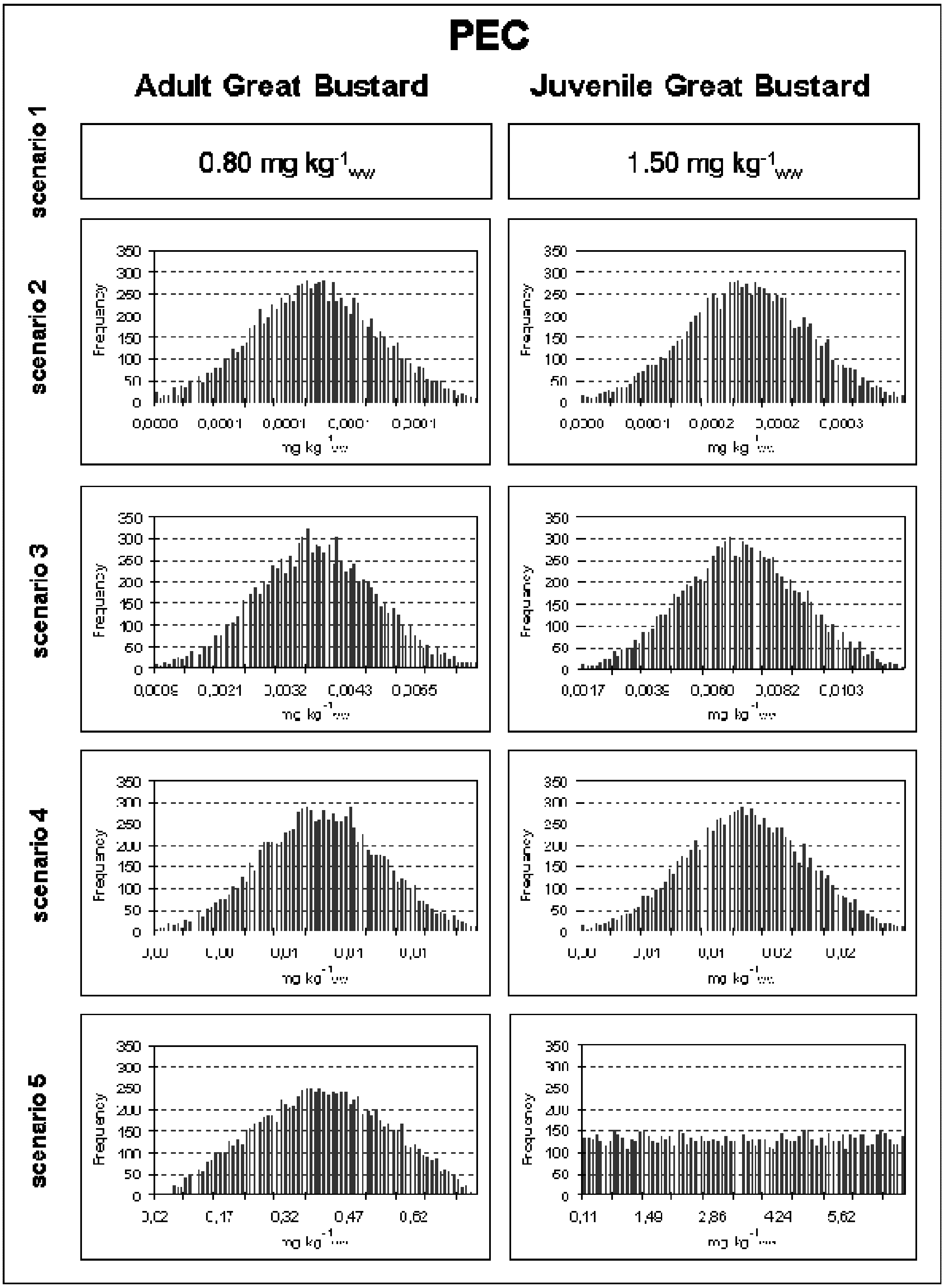


Figure 3.3. PEC (mg kg^{-1} wet weight) for Adult and Juvenile Great Bustards (*O. tarda*) in the five addressed scenarios.

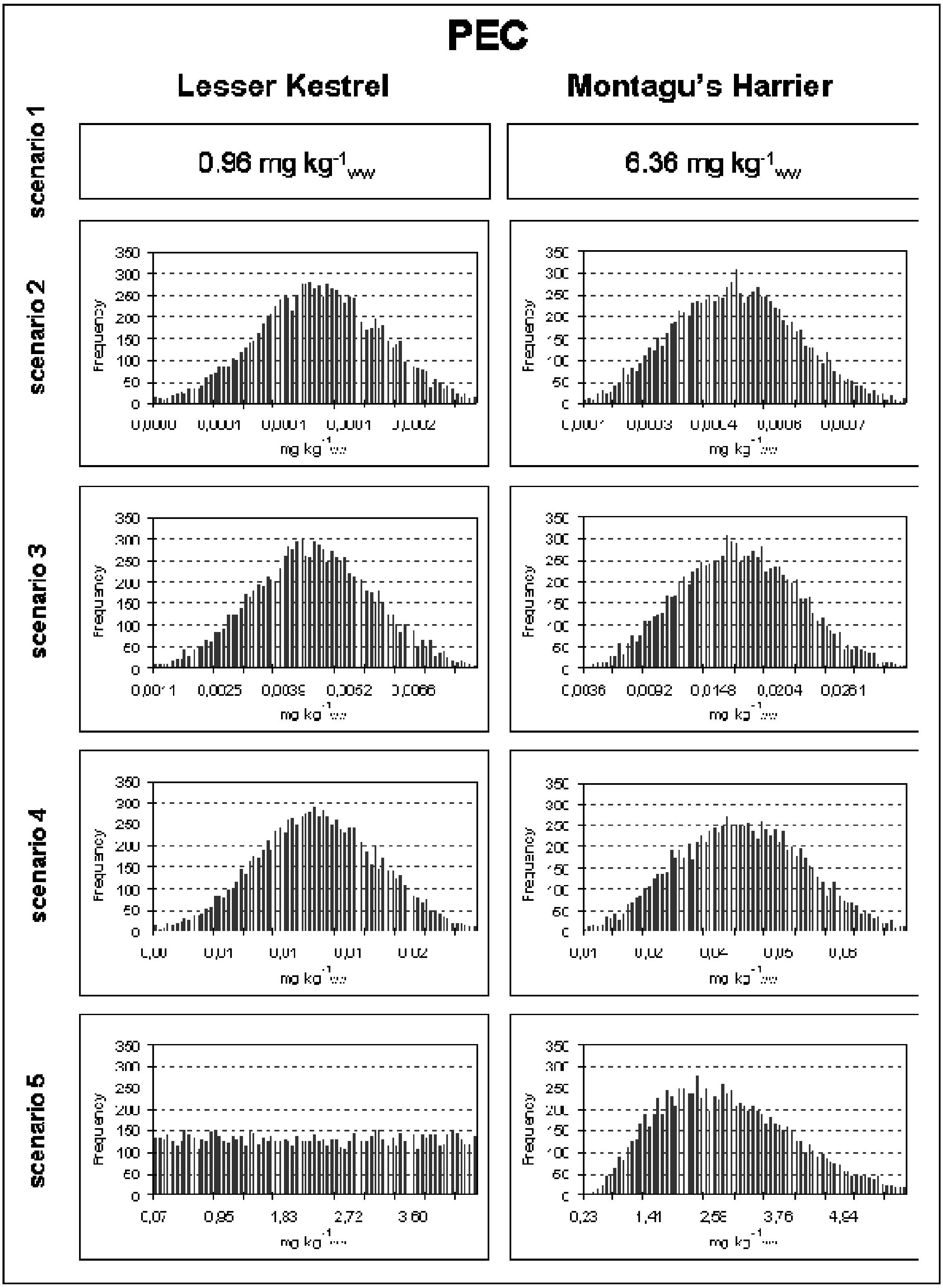


Figure 3.4. PEC (mg kg^{-1} wet weight) for Lesser Kestrel (*F. naumanni*) and Montagu's Harrier (*C. pygargus*) in the five addressed scenarios.

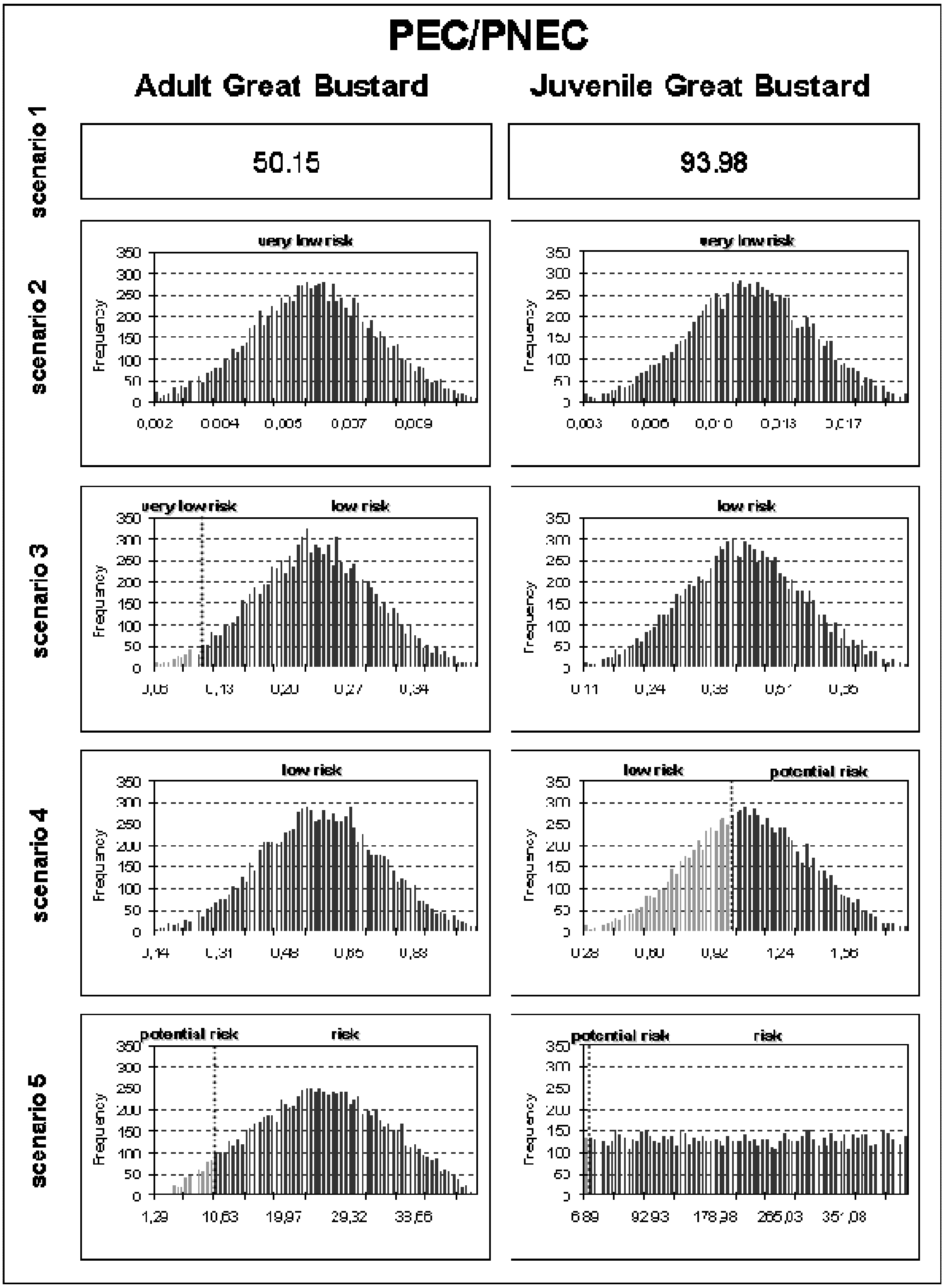


Figure 3.5. Risk characterization (PEC/PNEC) for Adult and Juvenile Great Bustards (*O. tarda*) in the five addressed scenarios.

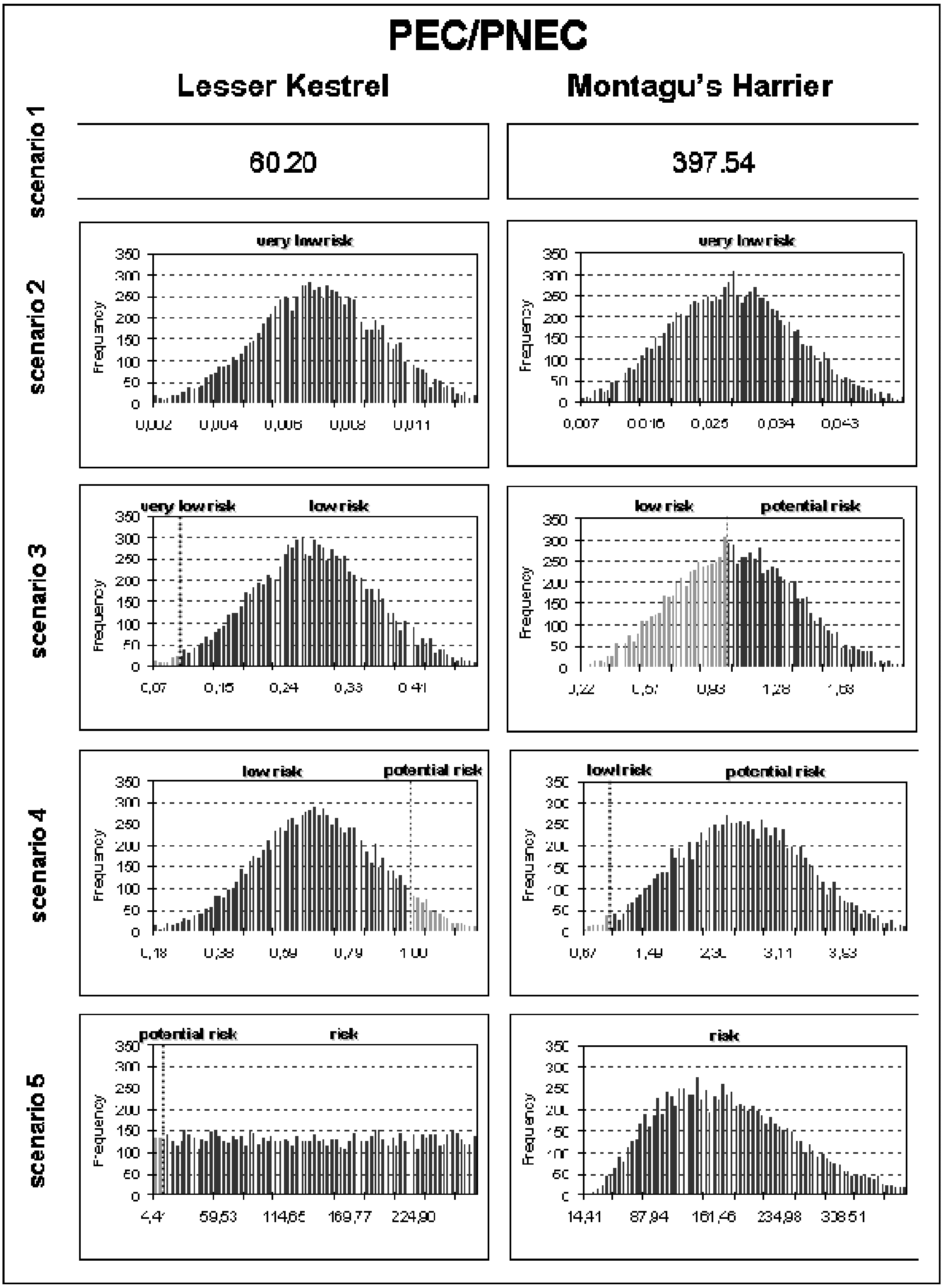


Figure 3.6. Risk characterization (PEC/PNEC) for Lesser Kestrel (*F. naumanni*) and Montagu's Harrier (*C. pygargus*) in the five addressed scenarios.

Risk Characterization

The characterization of risk is based on the comparison of the exposure (PEC) against the effects (PNEC) with the application of safety factors to account for uncertainty [49]. In the case of deterministic approach if the PEC/PNEC ratio is higher than one it is assumed to exist potential risk for the targeted organisms. The first scenario (figures 3.5 e 3.6), considering a worst case where plants are sown directly in the sewage sludge without mixing with agricultural soil, seems to pose risk for birds and particularly to the Montagu's Harrier where the PEC/PNEC ratio is one order of magnitude higher than in the other species.

For the characterization of risk with the probabilistic approach four classes of risk were defined (figures 3.5 e 3.6): (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. Furthermore the frequency distribution allowed the quantification of probability of risk classes with Crystal Ball software [38]. In scenario 2 a very low risk was assessed for all bird species. The third scenario led to an increase in the PEC/PNEC ratio moving the assessment to the class of low risk, remaining a 2.48 % and 1.21 % probability of very low risk in adult *O. tarda* and *F. naumanni*, respectively. But in the case of *C. pygargus* a potential risk of 56.37 % was also obtained. The increase in Cd concentration of soil in scenario 4 resulted in a slight moving forth of the risk classification. While only low risk was assessed for adult Great Bustard, a potential risk due to Cd was found in other birds (6.6 % Lesser Kestrel, 60.59 % juvenile Great Bustard and 98.65 % Montagu's Harrier). In the last assessed scenario the concentrations used were the ones from the microcosms experiments but unlike scenario 1, measured data for PEC in plants and invertebrates was used instead of calculations of exposure with BAF values. Nonetheless for small mammals BAF values were used for modelling PECs. By integrating the time dimension in the assessment as linear distribution of increasing concentrations in *E. andrei*, *C. arietinum*, *T. aestivum*, *R. sativus* and *S. gregaria*, the continuous uptake of Cd was modelled from day 0 (considering baseline concentrations) to the higher concentrations measured at day 28. As in PEC graphs, PEC/PNEC ratio reflect the patterns of the assumptions distributions in plants and invertebrates, especially for birds depending on the soil-plant-locust pathway. In juvenile Great Bustard and Lesser Kestrel there is an uniform distribution of the probability of risk, ranging from potential risk (lower than 10) and, respectively, 98.39 %

of risk reaching values higher than 351.08 and 97.02 % of risk reaching values higher than 224.90.

Discussion

Cadmium is a toxic, nonessential, trace metal that from soil can be rapidly transferred to plants [45; 50], and to invertebrates [44; 46] and small mammals [47; 48] through the food chain. In wild birds, Cd has been measured in raptors [51] and in a grouse species [52], though in low concentrations, showing the possibility of contamination in these animals due to different pathways.

The tested concentrations of Cd seem to be non-toxic for plants and invertebrates, in terms of seedling emergence for *C. arietinum* ($F_{1,30}$ = 0,0826; p = 0.776), *T. aestivum* ($F_{1,30}$ = 1.786; p = 0.191) and *R. sativus* ($F_{1,30}$ = 0,0882; p = 0.769), and in terms of mortality for *E. andrei* ($F_{1,22}$ = 0.0445; p = 0.835) and *S. gregaria* ($F_{1,30}$ = 0; p = 1). In fact, according to the Cd RAR, the Effect Concentration for 50 % (EC_{50}) of an *E. andrei* population (for a 84 day test in a pH 6.3 soil) was of 253 mg kg $^{-1}$, and the median EC_{50} for plants is about 100 mg kg $^{-1}$ [8]. The present worst case deterministic calculations for PEC in earthworms and plants is bellow these thresholds: *C. arietinum*, 0.72 mg kg $^{-1}$; *T. aestivum*, 5.73 mg kg $^{-1}$; *R. sativus*, 4.13 mg kg $^{-1}$; and *E. andrei*, 17.40 mg kg $^{-1}$. A previous study in a Cd contaminated grassland ecosystem revealed concentrations for a grasshopper species (2.4 mg kg $^{-1}$) [44] and an herbivorous (less than 10 mg kg $^{-1}$) and an insectivorous mammal (over 70 mg kg $^{-1}$) [47] at the same order of magnitude than the presently calculated PECs, respectively, 3.68 mg kg $^{-1}$, 3.15 mg kg $^{-1}$ and 104.55 mg kg $^{-1}$. But the increase in the considered trophic levels from adult Great Bustard, to juvenile Great Bustard, to Lesser Kestrel, and to Montagu's Harrier makes clear the contribution of diet and its consequences in risk characterization.

The quantification of probability of risk was possible with the Monte Carlo analysis in scenarios 2 to 5. Sewage sludge amended to agricultural soil in Castro Verde does not seem to pose risk to protected bird species from the SPA assuming a homogeneous distribution of the sludge within the soil, as the Cd concentration in the applied sludge is very low. However, increasing Cd concentrations to the maximum limit permitted by the Sewage Sludge Directive 86/278/EEC [7] (and national legislation, n.º 118/2006 [34]) settles a high probability of potential risk to *C. pygargus*. Furthermore the scenario for Cd concentrations in soil foreseen at the regional level by the RAR [8], that matches the baseline concentration in Castro Verde agriculture soils, also poses potential risk for Lesser Kestrel and particularly to juvenile Great Bustard and Montagu's Harrier.

The ecological parameters and particularly the diet of birds implies differences in the exposure to Cd, as also shown by the probabilistic model developed by Jongbloed *et al.* [53], and hence to the characterization of risks. *C. pygargus*, top predator feeding of all levels of the food chain with organisms with high BAF values, has the most critical food chain for secondary poisoning. Juvenile *O. tarda* and *F. naumanni* share the same food chain but since the first has a higher food intake rate per body weight shows a more critical food chain. Adult *O. tarda* that feeds only on plants has the less critical food chain.

Acknowledgments

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Annex 3.

Table A.3.I. Daily food intake rate per body weight, for wild birds and mammals from the conceptual model. Appendix I, EC (2002)[1].

	Body weight (g)	DEE Equation	DEE (Kj d^{-1})	Food characteristic Food type	Energy (kJ g^{-1}_{dw})	Moisture (%)
Adult Male Great Bustard	16000	Other birds	8387,7	Grasses, cereal shoots	18	76,4
Adult Female Great Bustard	3500	Other birds	2593,5	Grasses, cereal shoots	18	76,4
Juvenile Great Bustard	1500	Other birds	1348,0	Arthropods	21,9	70,5
Lesser Kestrel	200	Other birds	284,4	Arthropods	21,9	70,5
Montagu's Harrier	345	Other birds	433,3	90 % bird and mammls 10 % Arthropods	22,5	69,0
Herbivorous small mammals	25	Other eutherians	67,8	Grasses, cereal shoots	18	76,4
Insectivorous small mammals	10	Other eutherians	35,6	Soil invertebrates	19,3	84,6

	Assimil Effic. Food type	%	FIR (fresh material) (g day^{-1})	FIR / bw
Adult Male Great Bustard	Gruiformes Herbage	59	3346,6	0,209
Adult Female Great Bustard	Gruiformes Herbage	59	1034,8	0,296
Juvenile Great Bustard	Gruiformes Animal	34	613,7	0,409
Lesser Kestrel	Falconiformes Animal	84	52,4	0,262
Montagu's Harrier	Accipitriformes Animal	82	75,6	0,219
Herbivorous small mammals	Crops, forbs, mixed vegetation	74	21,6	0,863
Insectivorous small mammals	Insects	88	13,6	1,359

[1] EC (2002). *Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC.* SANCO/4145/2000 - final. Brussels: Europe Commission. Directorate - General Health and Consumer Protection.

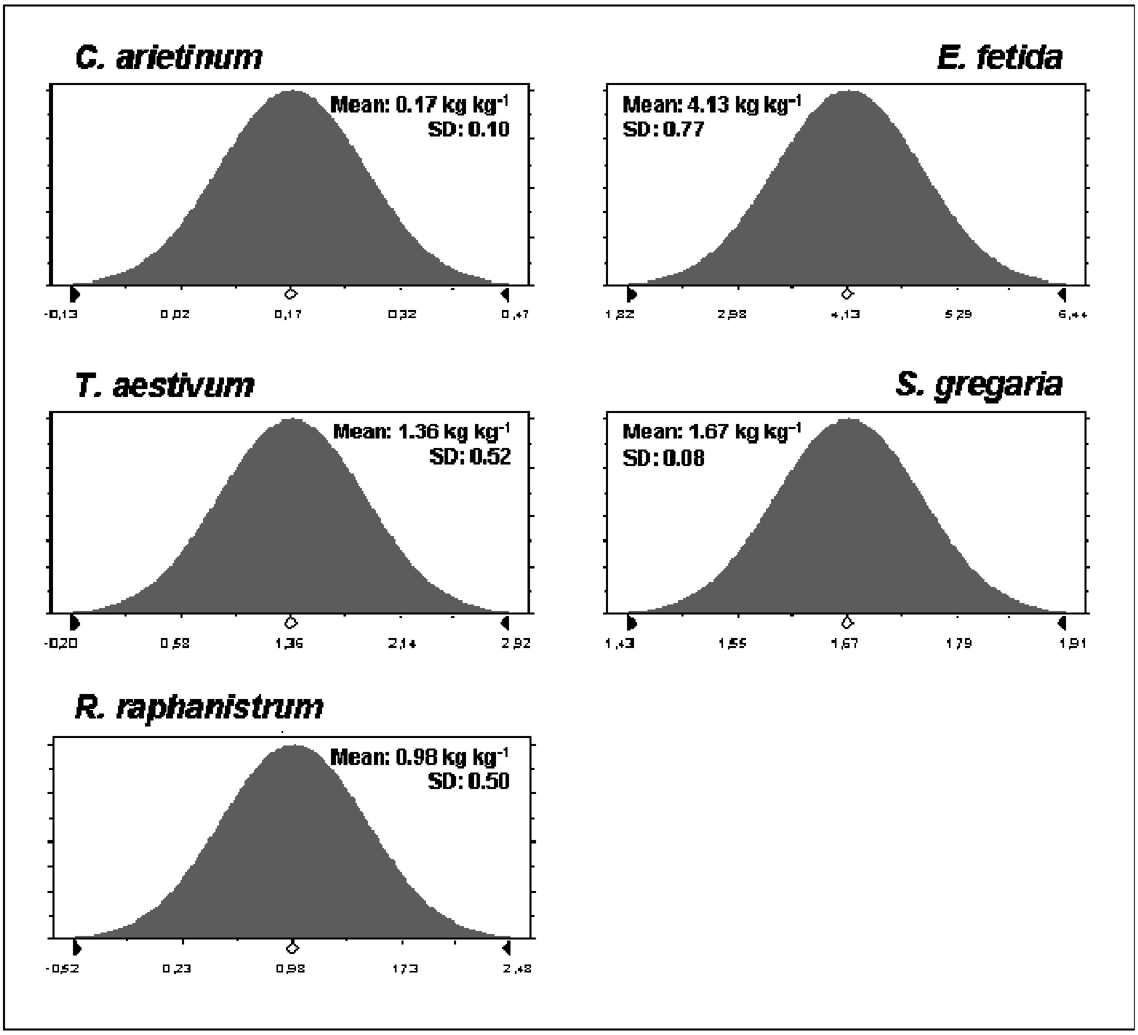


Figure A.3.1. Normal distribution assumptions for the plants and invertebrates BAFs of scenarios 2 to 4.

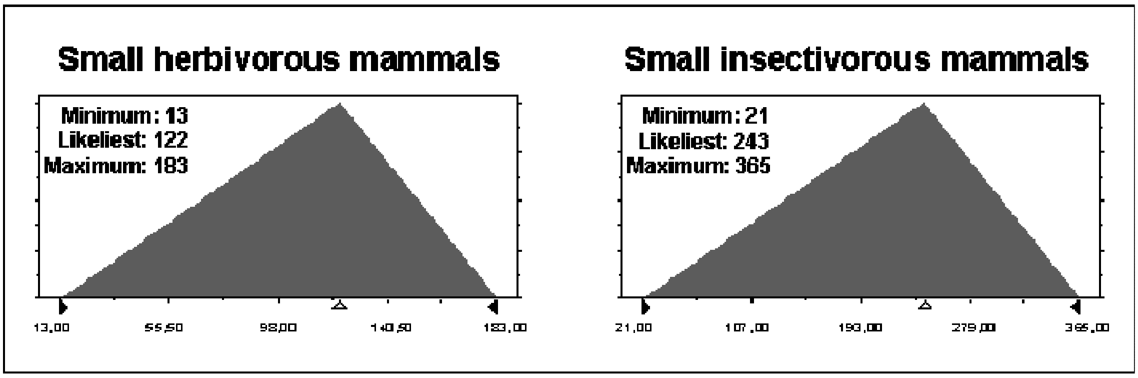


Figure A.3.2. Triangular distribution assumptions for small mammals age populations' (in days) of scenarios 2 to 4. Minimum value is the weaning period, the likeliest age corresponds to two thirds of the lifespan, and the maximum value stands for lifespan.

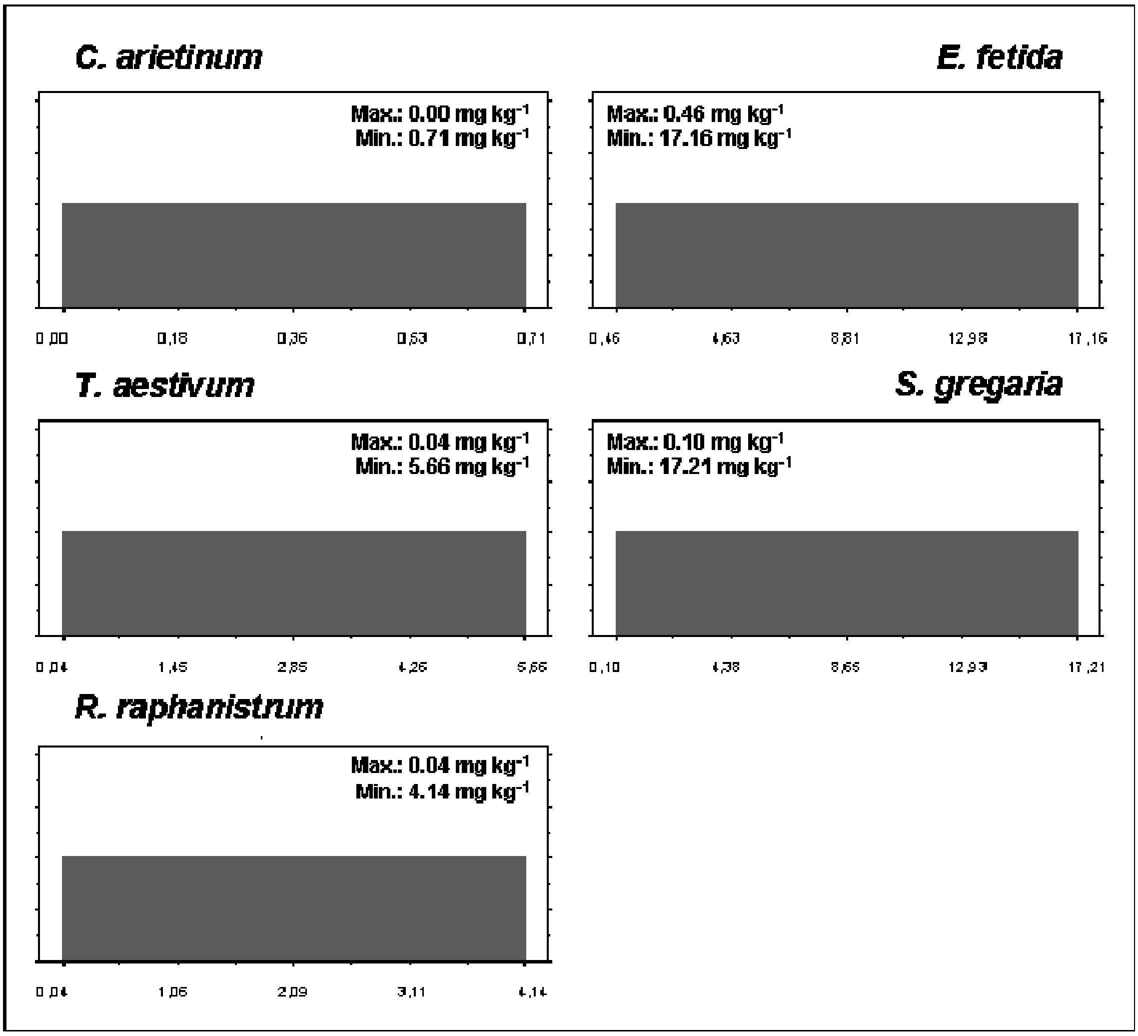
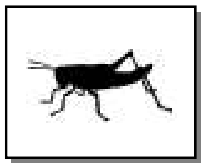


Figure A.3.3. Uniform distribution assumptions for inscreasing Cd concentration in plants and invertebrates, due to uptake over time, of scenario 5.

CHAPTER 4



Chapter 4. Assessing the risk of glyphosate and LAS in an European Mediterranean protected area

Abstract

European species depend to a large extent upon habitats created by man. The traditional forms of agriculture are essential for the conservation of many species, particularly birds. The main landscape of the bird special protection area (SPA) of Castro Verde, integrated in the European Natura 2000 Network, is characterized by extensive farmland of cereals and fallow land. But the traditional scheme of agriculture may still be responsible for the input of toxic chemicals like herbicides and the complex burden of sewage sludge used as soil fertilizer. The herbicide glyphosate (along with its major breakdown product AMPA) and the surfactant LAS, a major organic contaminant present in sewage sludge, were assessed for risks posed to birds of conservationist concern: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). Probabilistic approach for risk refinement was used. Real-case scenarios were used for exposure of organic contaminants in soil. While a potential risk of secondary poisoning is expected for birds that have a food chain based in sludge-amended agricultural soils, only a potential risk for juvenile Great Bustards is posed by herbicide usage according to the agricultural application rate.

Keywords: probabilistic risk assessment, protected area, agriculture, herbicide, glyphosate, sewage sludge, LAS, *Otis tarda*, *Falco naumanni*, *Circus pygargus*.

Introduction

Since the last glaciations human activity has shaped landscape across Europe and most of the continent surface has been used for producing food and timber or providing space for living. Therefore European species depend to a large extent upon landscapes created by man. One of the dominant land uses in the EU is the farmland (arable land and permanent grassland) that covers more than 45 % of the territory. The traditional forms of agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [1]. Following the overall trend, biodiversity in Europe's farmland has declined strongly in the last decades with a special emphasis to bird populations [2]. The most biodiversity-rich areas within agricultural landscapes are defined as High Nature Value (HNV) farmland. Greece, Portugal and Spain were the countries from EU-15 that had higher share (over 30%) of HNV farmland area of the total utilised agricultural area [3]. These areas are mainly found in the Mediterranean region and are strongly correlated with extensive farming systems. The intensification of agriculture, and concomitant increase in nutrient and pesticide inputs, has been identified as a major vector to the decrease of biodiversity [4]. The depleting role of herbicides to biodiversity is widely recognized (e.g. [5-9]). Sewage sludge has been used in agriculture as a source of nutrients for fertilizing and soil amendment, but at the same time it may be responsible for the input of toxicants in terrestrial compartment [10]. Recently, the concern on the presence of micropollutants in the sludge has been extended to organic chemicals [11]. A large list of chemicals used in consumer products can be found in the sludge. Detergent components are of special concern in countries such as Denmark [12].

The basis for action of the EU's Biodiversity policy is provided by the Birds and the Habitats Directives, the so-called "nature directives". Across Europe, several sites are classified under the nature directives, Special Protected Areas (SPAs) for wild birds [13] and Sites of Community Interest (SCIs) for habitats and endangered species [14], encompassing the Natura 2000 Network. In December 2006 it already covered more than 20 % of EU-25 territory [15].

The main objective of the present work is to assess the risk of two organic xenobiotics, glyphosate (herbicide) and linear alkylbenzene sulphonate LAS (anionic surfactant, present in sewage sludge), to protected bird species of a Portuguese bird SPA.

A conceptual model based on the food chain of the ecological receptors [CHAPTER 2] will be used to test the hypothesis that the organic pollutants reaching soil are uptaken by plants and then undergo biomagnification and be responsible for secondary poisoning of the target bird species. Probabilistic tools will be used for the refinement of risk assessment.

Methodology

Case study: SPA of Castro Verde

The present risk assessment will consider the SPA of Castro Verde in southern Portugal, Alentejo, as the case study for a farmland site from Natura 2000 Network. Landscape is characterized by a mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [16; 17]. In terms of climatic environment, over the last forty years, average seasonal weather conditions were as follows (temperature, rainfall): Autumn, 18 ºC, 200 mm; Winter, 15 ºC, 200 mm; Spring, 24 ºC, 120 mm; Summer, 31 ºC, 30 mm, [18]. Extensive agriculture of cereals with fallow and climatic conditions (hot, dry summers, and cool, wet winters) are responsible for bringing forth a steppic habitat characteristic of the most important Portuguese refuge for several bird species of conservationist concern like the Great Bustard (*Otis tarda*) [19], the Lesser Kestrel (*Falco naumanni*) [20], or the Montagu's Harrier (*Circus pygargus*) [21].

Table 4.I. Characterization of agriculture soil from the SPA of Castro Verde

Pedological descriptors			Physical-chemical properties		
Coarse sand	23.43	%	pH	6.1	
Fine sand	36.96	%	Residual humidity	4	%
Silt	24.87	%	Density	1.21	
Clay	14.76	%	Maximum water holding capacity	27.55	%
			NH_4^+ content	1	ppm
			Oxidizable C	1.74	%
			Total organic matter	3	%
			Extractable P	60	ppm
			Extractable K	98	ppm

Soil from the top 10 cm layer, was collected from a site of the “Herdade de Vale Gonçalinho” (N 43º 14’21 6’’, W 8º 30’35 3’’) that had not received sewage sludge or herbicide applications during the last decade, and was sieved *in situ* with a 4 mm mesh. Basic pedological descriptors and physical-chemical properties were analyzed following the British Society of Soil Science methodological procedures [22] (table 4.I).

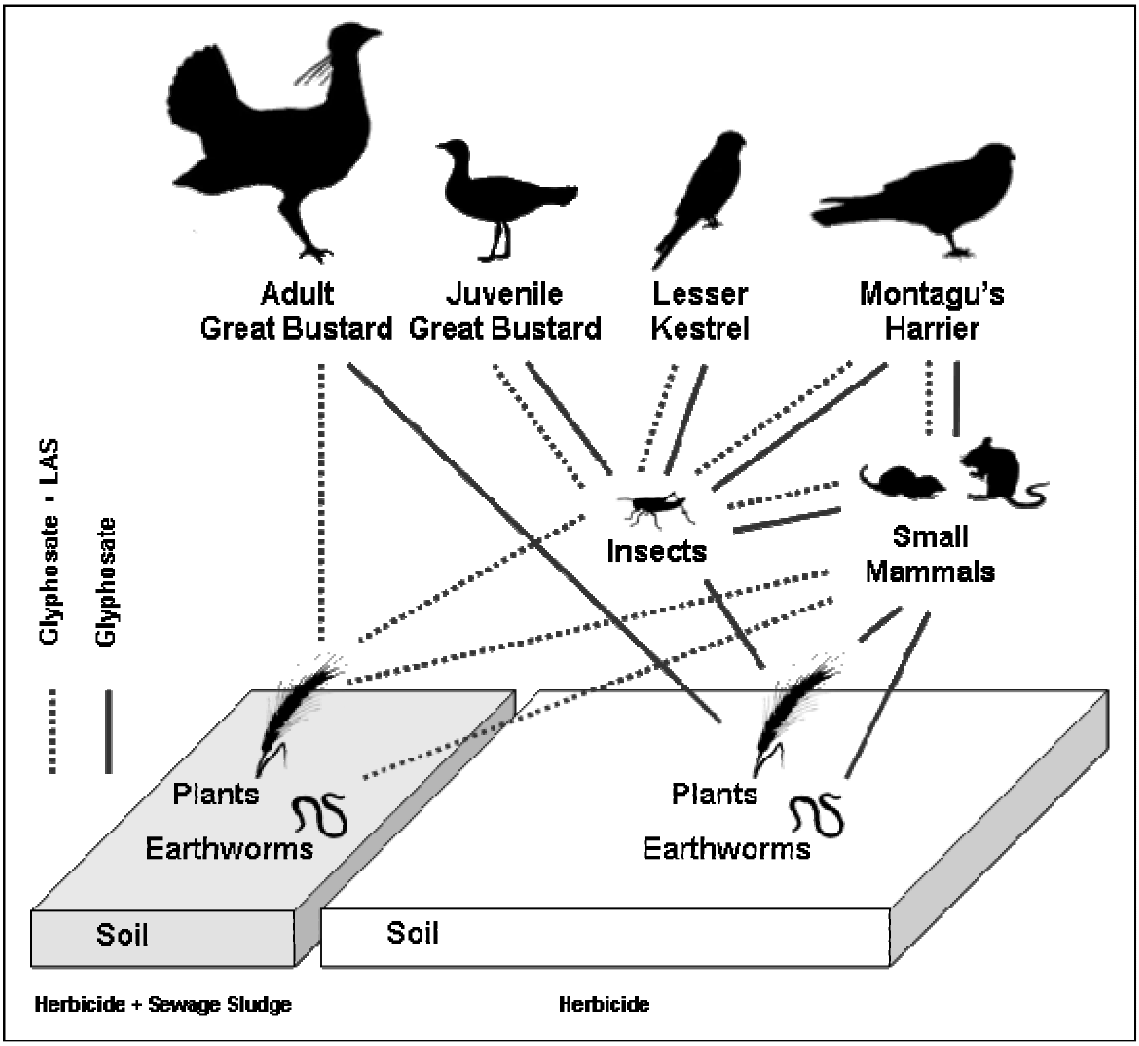


Figure 4.1. Conceptual model for LAS and Glyphosate pathways to the ecological receptors of a food chain from an extensive agriculture habitat (Adapted from CHAPTER 2).

Conceptual Model

The conceptual model (figure 4.1) used to assess the risk of the toxicants from extensive agriculture was described elsewhere [CHAPTER 2]. In short it is based on the effects of secondary poisoning along the trophic chain determined by the diet of the Great Bustard and two raptors, the Lesser Kestrel and the Montagu's Harrier.

In the present experimental work the two organic pollutants assessed with this model were:

(i) Herbicide glyphosate that is marketed as a non-selective, broad-spectrum, post-emergence herbicide [23; 24] and is applied in this farmland area before seedling.
(ii) Linear alkylbenzene sulphonate (LAS) that is the most widely used anionic surfactant in cleaners and detergents and is a major organic contaminant present in sewage sludge [25]; sewage sludge was deployed in limited 2 ha areas from the agriculture fields of the SPA of Castro Verde until 2002 in a program aiming to prevent desertification and soil erosion [26].

Once the organic toxicants reach plants through soil, they may be transferred along the food chain and reach the different considered ecological receptors, depending on the birds diet: from plants, organic pollutants may be bioaccumulated in adult Great Bustards, insects or small herbivorous mammals; small insectivorous mammals may accumulate toxicants from soil dwelling invertebrates e.g. earthworms; from insects, organic pollutants may be uptake by the animals that feed upon them, juvenile Great Bustards, small insectivorous mammals and both birds of prey; from small mammals, organic pollutants may reach the Montagu's Harrier. Hence, assuming that organic toxicants bioaccumulate along the food chain it may be expected higher concentrations in top predators.

Experimental set-up

The role of plants and invertebrates in the transfer of toxicants through the food chain was studied using terrestrial microcosms as surrogates of the ecosystem from the SPA of Castro Verde. For the purpose equipment from terrestrial model ecosystems (TME), field validated and ring-tested in an EU project [27] ("The use of TME to assess environmental risks in ecosystems", Project No: ENV4-CT97-0470), was used. But instead of enclosing intact soil-cores as a mesocosm approach [28] homogenised sieved soil was used instead, as proposed for Multi-Species Soil Systems (MS·3) microcosms [29], thus allowing better reproducibility [29; 30] and overcoming mesocosms' high variability [31]. Plants from the SPA were chosen in accordance to the Great Bustard feeding preferences [32-34]. Two crop species, common wheat (*Triticum aestivum*) and chickpea (*Cicer arietinum*), were studied. According to the traditional rotation scheme, *T. aestivum* is sown as a primary cereal in September-November and harvested in June-July, stage when leguminous crops (*C. arietinum*) are sown in smaller amounts [35]. Wild radish *Raphanus raphanistrum*, is a native weed from the Mediterranean region that has been reported to be resistant to herbicides [36], and its presence has been shown to reduce wheat above ground biomass due to its competitive annual growth habit and high fecundity [37; 38]. But since no commercial seeds of wild radish were available cultivated radish (*Raphanus sativus*) was used instead. Furthermore belonging to the same genus these two species can easily hybridize [37]. Earthworms play an important role in secondary poisoning of small vertebrates [39] and water infiltration and storage and soil aeration [40] thus contributing for the mobilization of toxicants [30]. *Eisenia andrei* cultured were in our lab were tested in the microcosms experiments. Orthoptera were chosen as test insects because they are important food items for juvenile *O. tarda* [33] but also to *F. naumanni* [41] and to a less extent to *C. pygargus* [42]. The locust *Schistocerca gregaria*, late first or early second instars, were acquired from Blades Biological Ltd (http://www.blades-bio.co.uk/) and left to acclimate to laboratory testing conditions during one week while fed *ad libitum* with dry bran and fresh grass.

The day before the beginning of the experiment, 10 kg of soil from the SPA of Castro Verde, saturated with 1.3 l of distilled water, was placed in 40-cm long high-density polyethylene columns (17.5 cm diameter) covered at the bottom with a plate of the same material and a thin inert gauze in between. In the case of treatment columns LAS was dissolved with the distilled water. A commercial formulation of LAS Ufasan 65 (CAS No: 25155-30-0), Unger Fabrikker A.S, with 65 % of active substance by mass and chains containing 11-12 carbon units, was tested. Soil samples were taken for LAS analysis. Columns were then placed in a cooled cart system allowing soil temperature to be at 12 ± 2 ºC. Afterwards Roundup Ultra (Bayer), the most used formulation of glyphosate, was sprayed on the top of soil treatment tubes.

Laboratory conditions were set to simulate climatic conditions of spring and autumn, respectively, when chickpea and common wheat are sown [18]. This meant an average temperature of 20 ± 2 ºC; air moisture inferior to 40 % and light/dark cycles 10/14 h. Lighting of 8000 lux intensity was provided by Philips SON-T Agro high pressure sodium lamps. To simulate rainfall, rain-heads made out of plexiglass (16.5 high and 14 cm diameter, with 12 evenly spaced holes where micro-pipettes are inserted) were placed above soil tubes twice a week with 1.2 l simulating monthly ca 55 mm rainfall per column. Six columns were allocated to the control and other six were allocated as treatment, per cart. Four carts were used giving a total of 24 control and 24 treatment replicates.

At day 0, 10 pre-weighted adult earthworms (with clitellum) were deployed as described in an international ring test for bioaccumulation of chemicals in earthworms [43], and 15 chickpea, 20 common wheat and 20 radish seeds were sown as proposed by OECD guidelines [44; 45], per soil column. Soil samples were taken for analysis of glyphosate and its major breakdown product AMPA (aminomethylphosphonic acid) allowing a 24-hour period for herbicide and its breakdown product to distribute along the soil column since it was not mixed with soil as in the case of LAS.

At day 14 three locusts were deployed per column; in one column per control and treatment in each cart, no locusts were deployed in order to evaluate plant growth and chemical concentration at the end of the experiment.

In the end of the experiment, day 28, aerial part of the plants was cut off and weighed; locusts were collected for survival assessment and the ones that survived were weighted and analysed for the presence of organic xenobiotics. The soil columns were removed from the cylinder and homogenised in order to take samples that were analyzed for humidity and LAS, glyphosate and AMPA. Earthworms were collected for survival assessment and the ones that survived were weighted and analysed for the presence of chemicals.

Chemical Analysis

Soil, plant and invertebrate samples were analyzed by terracon GmbH (http://www.terracon-jueterbog.de/) with HPLC-FLU/ELCD analogue DIN EN 38407-F22 in the case of Glyphosate and AMPA, and HPLC-UV acc. to internal method No.QA-TENS01/03 in the case of LAS.

Risk Assessment – scenarios and probabilistic assumptions

Since a site-specific risk assessment was intended, it was decided to use, as far as possible, realistic exposure levels of Roundup Ultra and LAS. In the case of the herbicide the application was made following the recommendation of Bayer Crop Science (http://www.bayercropscience.pt/) of a maximum agronomic application rate of 10 l ha^{-1} for pastures and wheat crops, which meant a volume of 24 µl per microcosm. The disposal of sewage sludge in agricultural soil in Castro Verde was preceded by chemical analysis but only metals were analyzed [26]. The present Portuguese regulation regarding the usage of sewage sludge in agriculture [46] foresees a LAS limit of 2600 mg $kg^{-1}{}_{dw}$ in sludge but until 2002 when the program aiming to prevent desertification and soil erosion [26] ended, organic compounds were not covered by national [47] or EU legislation [48]. Considering the predicted environmental concentrations (PEC) in sludge amended soils, modelled in household uses from the LAS risk assessment by HERA [49] of 5.6 mg kg^{-1}, and the range of 1-10 mg kg^{-1} referred to as a worst-case scenario with a dosage of 2 T ha^{-1} in a Danish Workshop on LAS risk assessment [12], a concentration of 10 mg kg^{-1} was tested in microcosms, knowing that in Castro Verde a mass of 5-6 T ha^{-1} at depth of 30-75 cm was amended [26].

PEC values of glyphosate, AMPA and LAS for plants and invertebrates were obtained from the measured concentrations in microcosm testing. For the calculation of PECs in small mammals and in target birds, formulas were adapted from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [50]. The bioaccumulation factors of organics in small mammals was obtained from the formula $BAF = \alpha * F / k_2$, where α is the fraction of ingested dose that is absorbed, F is the food intake rate per body weight (FIR/bw) (calculations followed information provided in appendix I of the referred guidance document), and k_2 is the rate constant for depuration. In the case of glyphosate $\alpha = 0.3$ (30 %) and $k_2 = 1$ (100 % at the end of 168 h) [24; 51]; for AMPA toxicokinetics can be characterized by $\alpha = 0.2$ (20 %) and $k_2 = 1$ (100% between 24 and 120 h) [52]; and for LAS values are as follows $\alpha = 0.85$ (80-90 %) and $k_2 = 0.63$ (60-65 %) [49]. PEC calculations for the ecological receptors are given by the formula: $PEC_{Bird} = (FIR/bw) * C * PD$, where C is the concentration of organic chemical in fresh diet and PD is the fraction of food type in diet [53]. Diet of small mammals was assumed to include equal proportions of the tested plant species but for adult Great Bustards, diet was considered to be constituted on the proportions described by Palacios *et al.* [32] for the plant families tested in microcosms experiments: Fabaceae (40 %) for *C. arietinum*, Brassicaceae (32 %) for *R. sativus*, and Poaceae (28 %) for *T.*

aestivum. In view of the diet described for *C. pygargus* by Corbacho *et al.* [42], the following percentages of food were considered for the PEC calculations: 70 % herbivorous small mammals, 20 % insectivorous small mammals, and 10 % locusts.

Risk characterization is based on the comparison between exposure and assessment but the way this comparison is formally conducted differs depending on the considered protocols [54]. EU guidelines for the assessment of plant protection products (e.g. [53]) compare toxicological endpoints (depending on target organisms) with exposure according to a Toxicity to Exposure Ratio (TER = Toxicity/PEC). In the approach for industrial chemicals proposed by the EU Technical Guidance Document on Risk Assessment [55] the assessment of effects is based on the establishment of Predicted No Effect Concentrations (PNEC's) and characterization is given by a PEC/PNEC ratio. According to the EU's Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) [54] the main advantage of TER comparisons lies on the possibility of evaluating the ecological relevance of the identified potential risk. Since the present model and assessment scenarios already cover the ecological specificities of the different elements of the trophic chain, additionally allowing the differentiation between bird species and even within the same species (juvenile and adult Great Bustard), and in order to standardize risk assessment the simplified comparison such as the PEC/PNEC ratio was used. For the effects assessment, PNECs were derived from literature ecotoxicological data for active substances and following the principles of the Technical Guidance Document on Risk Assessment [55] for the application of safety (assessment) factors due to secondary poisoning. Hence, from a no observed effect concentration (NOEC) for reproductive toxicity to birds at a concentration of 200 mg kg^{-1} glyphosate (assessment factor of 30) [51] a PNEC of 6.67 mg kg^{-1} was derived. In relation to AMPA a no observed adverse effect level (NOAEL) for maternal and developmental toxicity of 400 mg kg^{-1} bw day^{-1} in rat (Olson 1991 cit. in [52]) was converted (conversion factor of 10) into a 4000 mg kg^{-1} NOEC (subchronic test; assessment factor of 90), giving a PNEC of 44.44 mg kg^{-1}. Regarding LAS, a PNEC of 5.56 mg kg^{-1} was derived from a NOAEL of 50 mg kg^{-1} bw day^{-1} in rat [49] converted (conversion factor of 10) into a 500 mg kg^{-1} NOEC (duration of the test 90 days; assessment factor of 90). For characterizing the risk of glyphosate, AMPA and LAS an additional safety factor of 10 was included for covering the individual variability within vertebrate species, as the assessment focuses on defined species with ecological value which should be protected at least at the population level within the area. For transparency reasons this factor was not included in the PNEC derivation, but in the interpretation of the risk values. Thus, the PNECs were developed following the TGD recommendations, but the acceptability threshold for the PEC/PNEC was established as 0.1, instead of 1 which is the value recommended in the guidance document for generic assessments.

The refinement of risk assessment was done using the probabilistic approach with Monte Carlo analysis (10000 trials) performed with Crystal Ball software [56]. As assumptions, the concentrations measured in plants and invertebrates were given a normal distribution, and PEC and risk characterization (PEC/PNEC ratio) for target birds were defined as forecasts.

Results

Exposure

Glyphosate and AMPA

As a result of the agronomic application rate of Roundup Ultra, at the end of a 24-hour period a concentration of 0.02 mg kg^{-1} of glyphosate could be found in soil (table 4.II). But at the end of 28 days, the concentration of herbicide was below the quantification limit of the analysis equipment (< 0.01 mg kg^{-1}). The concentration in invertebrates was also below the quantification limit (< 0.1 mg kg^{-1}) but in the case of plants the uptake was extremely high when comparing concentration in *C. arietinum*, *T. aestivum* and *R. sativus* with soil concentration. The breakdown of glyphosate could be observed after 24 hours since the concentration of AMPA at day 0 was two orders of magnitude higher (33.62 mg kg^{-1}). At the end of the experiment, AMPA was still detectable in soil and the uptake occurred in both plants and invertebrates.

Since only in plants the uptake of glyphosate could be determined by analytical methods, the calculation of PEC was only done for herbivorous small mammals and for adult Great Bustard that feed on plants. For Montagu's Harrier PEC was also calculated since they feed on herbivorous small mammals to which BAF was calculated. The birds' exposure to glyphosate is laid down in table 4.III with the deterministic approach whereas the probabilistic distributions are shown in figure 4.2. Both bird species bioconcentrate glyphosate but adult Great Bustard, that feed only on plants, accumulate higher concentrations of herbicide; Montagu's Harrier prey on herbivorous small mammals that have BAF lower that one, hence lower concentrations of glyphosate reach the top predator. As the result of the Monte Carlo analysis and following the normal distributions assumed in the PECs for plants, exposure in birds presents a normal distribution, but the range of concentrations in adult Great Bustard is higher that in Montagu's Harrier.

Given that for AMPA the uptake could be determined in plants and invertebrates, PEC was calculated for all birds, as shown in table 4.III for the deterministic approach and in figure 4.3 with probabilistic methods. Adult Great Bustard and Montagu's Harrier, that bioaccumulate glyphosate, have concentrations of AMPA less than 1 mg kg^{-1}. While not bioaccumulating the parent compound, juvenile Great Bustard and Lesser Kestrel show higher bioaccumulation of AMPA. This fact may be explained by its diet based on locusts that bioconcentrate AMPA (BAF of 2.25 kg kg^{-1}). These differences on birds' exposure can also be observed from the Monte Carlo analysis along a normal distribution that reflects the assumptions for exposure in plants and invertebrates.

Table 4.II. Chemical concentrations (wet weight) in soil and in the organisms from the microcosms' experiments. Glyphosate and its breakdown product AMPA were analyzed in soil samples at day 0 and day 28. Soil samples were analyzed at day -1 and day 28 for the presence of LAS.

GLYPHOSATE concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day 0	0.02	-	-	-	-	-
Day 28	< 0.01	< 0.1	1.71	2.17	0.79	< 0.1

AMPA concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day 0	33.62	-	-	-	-	-
Day 28	19.59	4.80	2.88	5.97	1.62	10.80

LAS concentration (mg kg^{-1})						
	Soil	*E. andrei*	Plants *C. arietinum*	Plants *T. aestivum*	Plants *R. sativus*	*S. gregaria*
Day -1	10.13	-	-	-	-	-
Day 28	5.06	14.74	5.06	8.28	3.30	3.96

LAS

In the beginning of the experiment the concentration of LAS in soil was of 10.13 mg kg^{-1} but after 28 days it was reduced by half (table 4.II). Considering concentration in soil at day 28, bioconcentration took place in *E. andrei* and at a less extent in *R. sativus* and *C. arietinum*.

The different pathways consequential from the birds' diet results in PEC values of the same order of magnitude for all ecological receptors. This fact can be observed in both deterministic (table 4.III) and probabilistic approach (figure 4.4). Like in the Monte Carlo analysis performed for herbicide and its breakdown product, PEC values for LAS in target birds follow a normal distribution as a result of the assumptions for exposure in plants and invertebrates.

Table 4.III. Deterministic risk assessment of Glyphosate, AMPA and LAS for the target bird species: exposure assessment (PEC), effects assessment (PNEC) and risk characterization (PEC/PNEC).

	Glyphosate				
	Adult Great Bustard	**Juvenile Great Bustard**	**Lesser Kestrel**	**Montagu's Harrier**	
PEC	0.39	-	-	0.06	mg kg^{-1}
PNEC		6.67			mg kg^{-1}
PEC/PNEC	0.58	-	-	0.09	

	AMPA				
	Adult Great Bustard	**Juvenile Great Bustard**	**Lesser Kestrel**	**Montagu's Harrier**	
PEC	0.84	4.42	2.83	0.42	mg kg^{-1}
PNEC		44.44			mg kg^{-1}
PEC/PNEC	0.19	0.99	0.64	0.09	

	LAS				
	Adult Great Bustard	**Juvenile Great Bustard**	**Lesser Kestrel**	**Montagu's Harrier**	
PEC	1.35	1.62	1.04	1.82	mg kg^{-1}
PNEC		5.56			mg kg^{-1}
PEC/PNEC	2.43	2.91	1.87	2.28	

Risk Characterization

In the deterministic approach for risk characterization point estimates are obtained and when the PEC/PNEC ratio is lower than 0.1, risk is considered acceptable since an additional safety factor of 10 was included for covering the variability within bird species. From table 4.III it may be assumed that the utilization of glyphosate at agronomic application rates: poses an acceptable risk for Montagu's Harrier and a low risk for adult Great Bustard, Lesser Kestrel and juvenile Great Bustard although, for the last two low risk characterization is due to the metabolite AMPA because they do not uptake the parent compound. The present tested LAS concentration in soil shows a scenario with risk for all the considered protected birds from the SPA of Castro Verde.

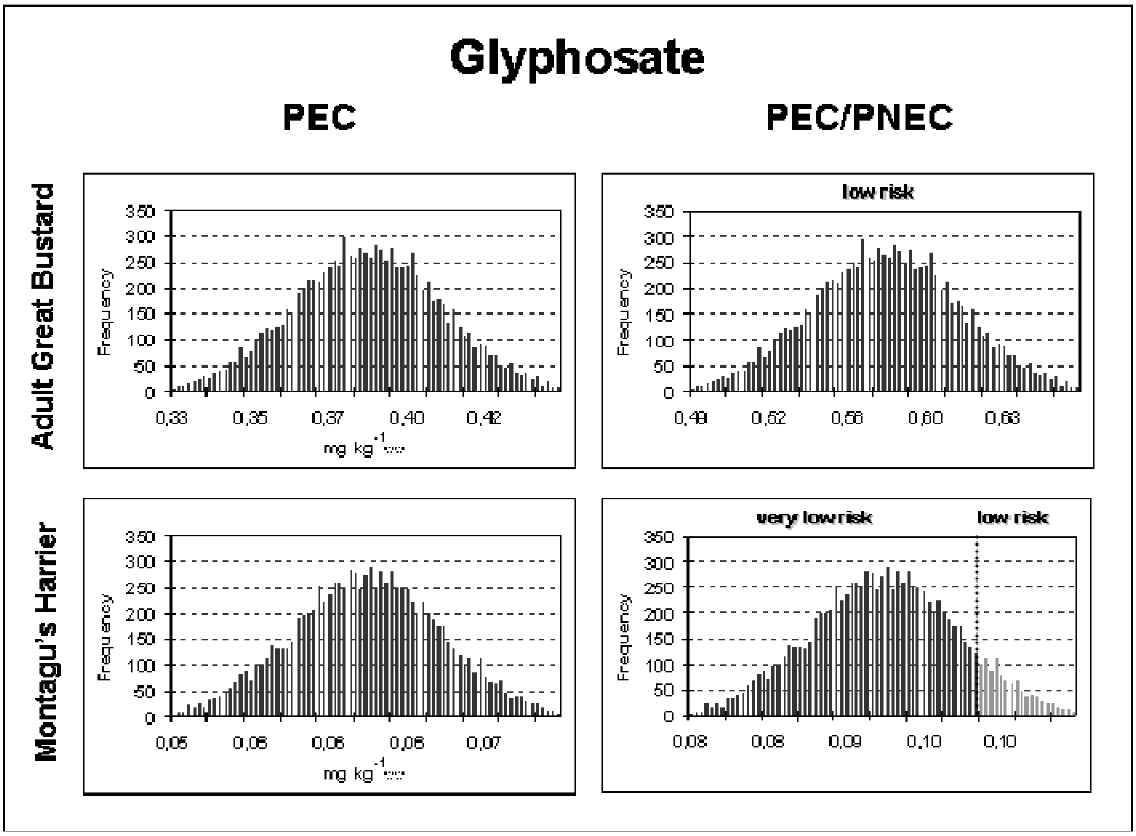


Figure 4.2. Probabilistic risk assessment of Glyphosate for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

The Monte Carlo analysis with Crystal Ball allows the forecast of the distribution of concentrations where the likelihood of the extent of a defined range may be quantified. Thus for the characterization of risk with the probabilistic approach four classes of risk were defined (figures 4.2, 4.3 and 4.4): (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. The risks from the usage of glyphosate, following the manufacturer suggested application rate, are considered to be: very low to Montagu's Harrier with a 20.94 % probability of low risk due to AMPA; low for adult Great Bustard and Lesser Kestrel; and low for juvenile Great Bustard but with a probability of 47.67 % of potential risk due to the degradation metabolite. The considered worst-case scenario for LAS concentration in sludge amended soils may pose a potential risk for birds of conservationist concern from the SPA of Castro Verde, namely *O. tarda*, *F. naumanni* and *C. pygargus*.

Discussion

For the assessment of glyphosate its degradation metabolite AMPA must also be taken into account and consequently when comparing the risk of the two compounds for the same bird, the higher level of risk must be considered. In the case of the Montagu's Harrier in spite of being the top predator hence expected to be exposed to higher concentrations of toxicants due to secondary poisoning through the food chain [54], glyphosate was assessed to be of acceptable risk or of an overall very low risk according to the probabilistic distribution. This fact may be explained by the low BAF calculated for small mammals (BAF in small herbivorous mammals: 0.26 glyphosate and 0.17 AMPA; BAF in small herbivorous mammals: 0.41 glyphosate and 0.27 AMPA) that constitute the main diet of this raptor [42]. The risk of glyphosate may be considered to be low for adult Great Bustard. Considering the unmetabolized parent glyphosate, there was no exposure, i.e. no contact between stressor and receptor, for juvenile Great Bustard and Lesser Kestrel since it was not bioaccumulated in their food items, i.e. locusts. But the breakdown product AMPA was accumulated along the food chain though posing low risk to *F. naumanni* but potential risk to juvenile *O. tarda* like the hawk feeds on locusts but has higher feeding rates per body weight. It is clear the advantage of assessing the risk by means of probabilistic methodologies since according to the determinist approach only a low risk could be observed for juvenile Great Bustard but the exposure distribution showed almost 50 % of probability of potential risk of glyphosate degradation products, despite being indicated as a low toxic substance to vertebrates [52]. The effects on juvenile individuals have consequences at the turnover of the population thus jeopardizing the conservation of the species.

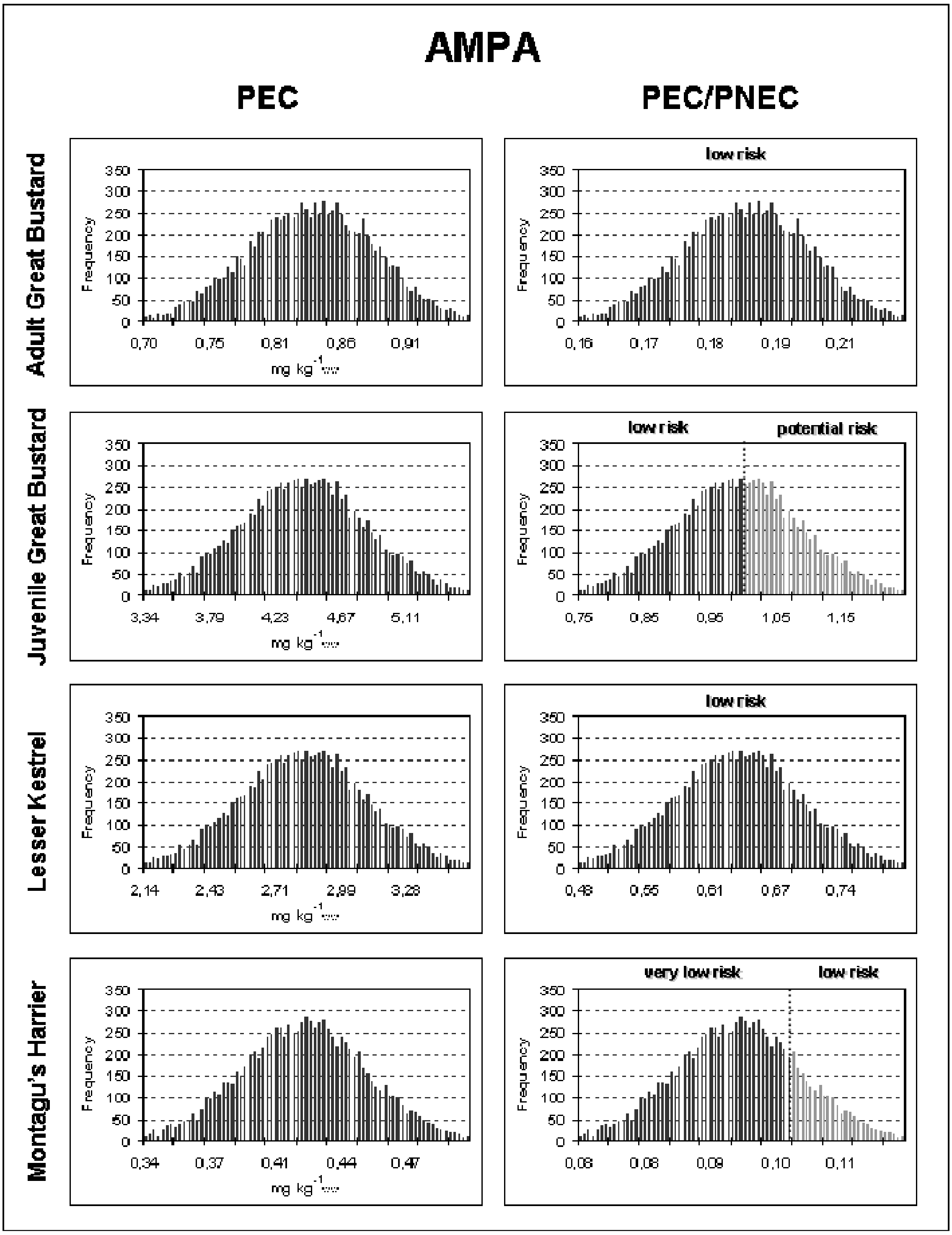


Figure 4.3. Probabilistic risk assessment of AMPA for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

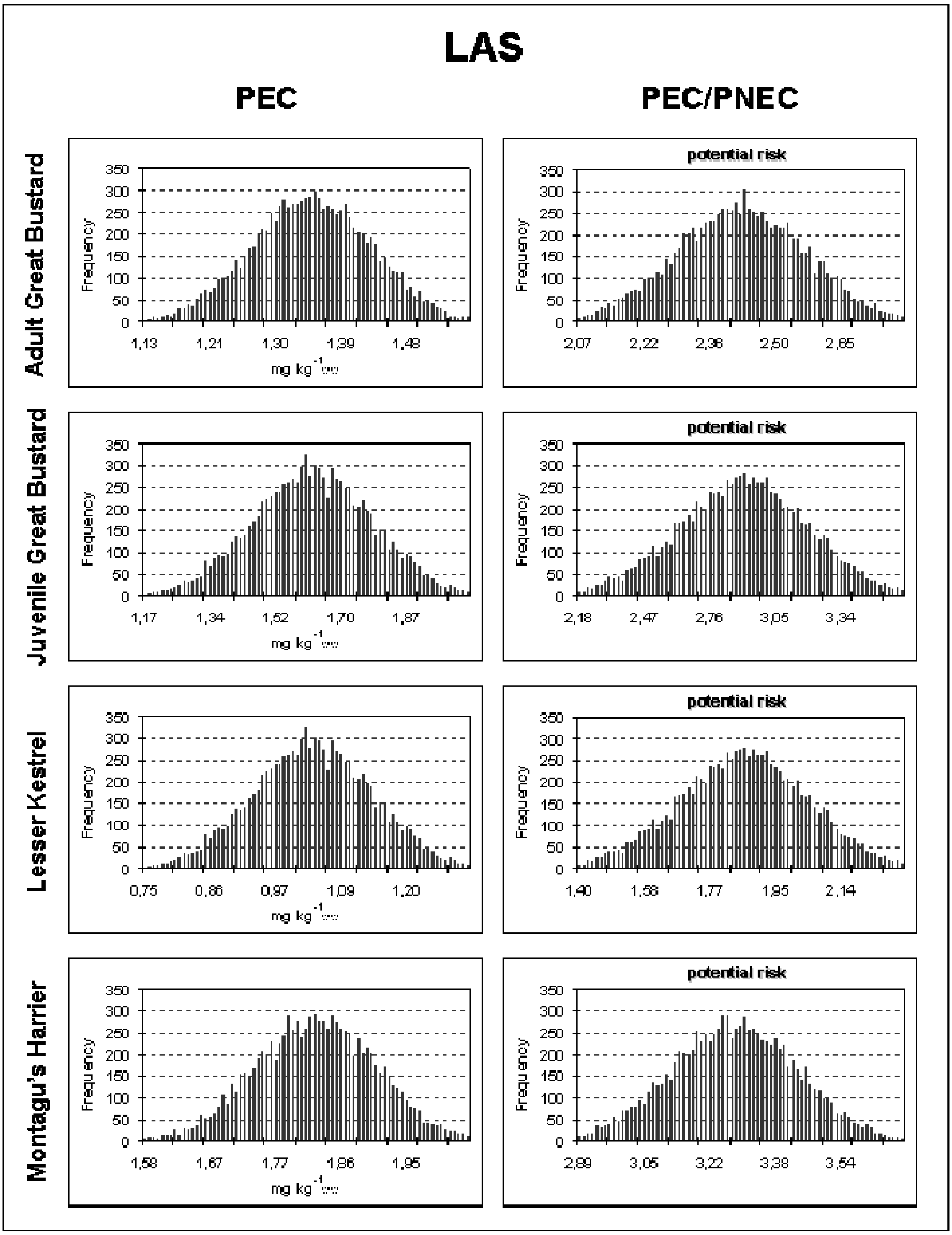


Figure 4.4. Probabilistic risk assessment of LAS for the target bird species: distribution of exposure assessment (PEC), and risk characterization (PEC/PNEC).

Some of the main facts referred in literature to explain why LAS is not dangerous to the environment are the evidence that it is readily degraded by aerobic microbial processes and does not bioaccumulate [57; 25; 49]. In fact some studies show that LAS is not even uptake by plants [12]. This may also be explained by the fact that it binds strongly to organic matter due to its negative charge [11]. Furthermore there is a common agreement on the fact that LAS has no risk for terrestrial (and aquatic) compartment as assessed by HERA [49] and the occurrence of bioconcentration is highly unlikely with an extremely low potential for secondary poisoning [58]. Nonetheless the present experimental work with terrestrial microcosms and respective risk assessment indicate that LAS may bioaccumulate in plants and concentrate through the food chain, and be responsible for secondary poisoning, having a potential risk for the considered bird target species, as assumed in our initial hypothesis. The fact that the soil from Castro Verde has low organic matter content may account for plants and invertebrates uptake hence influencing its transfer along the food chain. The uncertainty remains on whether the chosen scenario for exposure, 10 mg kg^{-1} of LAS in soil, might have been too high but organic compounds were not analysed in the sludge amended in Castro Verde nor were references found for background concentrations of LAS in Portuguese sludge-amended agricultural soils.

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CHAPTER 5

Chapter 5. The perception of risks from extensive agriculture in a Nature 2000 Network site

Abstract

Risk communication stands after risk assessment procedure, as a step where the exchange of information takes place between scientific community and stakeholders. Therefore the perception of risks to the different target groups needs to be studied. The present paper describes an evaluation of the perception of the risk, to general public, farmers and local authorities, from extensive farming practices in a cereal steppe in Castro Verde, southern Portugal (Alentejo). This area is included in the European Nature 2000 Network as a special protection area (SPA) for wild birds in accordance to the Birds Directive (79/409/EEC) due to its importance in the conservation of protected species like the Great Bustard (*Otis tarda*). For the purpose a questionnaire-based survey was carried out in the municipality of Castro Verde. The agriculture sector showed respondents with higher percentage of academic degree (but also a significant part of the respondents had only attended primary school) and a better knowledge on precise aspects of the SPA. The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. The environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information about the SPA to the people from the region .This work is an important contribute for the development of a risk communication framework for risks posed by extensive agriculture in a Natura 2000 Network site.

Keywords: risk communication, risk perception, natura 2000 network, extensive agriculture.

Introduction

There is frequently a gap in communication between the knowledge obtained by the scientific community and the general public, which delays the prevention and resolution of environmental problems. Risk Communication is defined by the OECD [1] as the interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public. Thus communication is an important tool in understanding environmental problems and in the orientation of decision-making [2]. But in order to decisions being understood and accepted by potentially affected individuals or groups as well as the general public it is important that risk communication takes into consideration perception of people towards environmental risks. In Europe, Risk Assessment protocols are used to set the impact of chemical contamination on biota [3]. This scientific step underpins the decision-making process defined as Risk Management that involves considerations of political, social, economic, and technical factors [1]. Therefore risk communication is fundamental in enhancing the likelihood that risk management decisions will incorporate the results of the risk assessment [4].

Questionnaire-based surveys have been used by European institutions to determine how people perceive risk, being therefore a contribution to the development of policy initiatives and communication events related to risk issues [5; 6]. A similar endpoint to "perception of risk" is used to deal with the loss of biodiversity; public awareness was defined as an indicator for biodiversity in the "Proposal for a first set of indicators to monitor progress in Europe" by the European Environmental Agency, [7]. Of course public awareness is not the same as public perception but ultimately both concepts are addressed when aiming to induce a cultural change towards sustainability.

In the EU, a network of protected areas, Natura 2000 Network is being built on the designation of areas for conservation under the Birds and Habitats directives, the so-called "nature directives", that constitute the European policy basis for halting the loss of biodiversity. Once in Natura 2000 Network the conservation status of habitats and species listed in the directives must to be maintained favourable which means that specific management plans with necessary restrictions on activities carried out, within, and around sites must be defined by each Member State [8; 9]. One of the dominant land uses in the EU is farmland (arable land and permanent grassland) that covers more than 45 % of the territory. Traditional farming practises like extensive agriculture are essential for the survival of many species and their habitats. Moreover 50 % of all species in Europe have been estimated to depend on agricultural habitats [10]. In Portugal more than 25 % of Natura 2000 habitats depend upon the continuation of extensive farming practices whereas the average EU-15 value in 2004 was of 18 % [11]

The key objective of the present work is to contribute for the development of a risk communication framework for risks posed by chemicals associated to extensive agriculture practice in a Natura 2000 Network site. Thus we aimed to assess the awareness and perception of the risk, to general public, farmers as the major actors in the continuation of extensive agricultural practices, and local authorities and decision-makers.

Methodology

A questionnaire-based survey was performed in the Municipality of Castro Verde in southern Portugal, Alentejo, since it has the higher percentage of land (55 %) from a Special Protection Area (SPA) for wild birds with a total area of 79007 ha. This questionnaire aimed 3 different target groups: general population, people from the agriculture sector and servants from local authorities. The survey will provide information for a risk communication process subsequent to the assessment of risks posed to the ecosystem by extensive agriculture within the limits of the SPA.

Study area

The SPA of Castro Verde is characterized by extensive farm fields with no arboreal vegetation and some less representative habitats with no agricultural use such as shrublands (of scrub *Cister ladanifer*) and woodlands (mainly holm oak *Quercus rotundifolia* but also a few olive groves *Olea europea*). This Mediterranean cereal steppe habitat is the refuge for some bird species of conservationist concern like the Great Bustard (*Otis tarda*) [12]. The traditional soil use creates a landscape mosaic of cereal fields, stubble, ploughed fields, and fallow land that is frequently used as pasture for sheep [13; 14].

The Questionnaire

The survey aimed to get a general picture of perceptions and views among the different stakeholders from the SPA of Castro Verde. Knowing that respondents would have different cultural and professional backgrounds, the questions selected tried to be as unambiguous and simple as possible, and an overall effort was made to make the questionnaire absolutely understandable.

After a brief introduction and invitation to participate, a group of questions were set to assess the socio-demographics of the respondents (Annex 5). The questions that followed were based on a previous survey conducted within an ongoing project on Global Sustainability Assessment in a Spanish SPA (Ramos M. J., personal communication) with a similar habitat and populations of birds from the same species. Firstly we wanted to know how far people were informed about the SPA, e.g. its ecological values, and who provided them with that information. Finally it was intended to gain inside on what people think about the factors that underpin the conservation of the SPA, and in the last question respondents were asked to reflect about the risks of a series of hazardous activities.

Target groups

It was decided not to carry out conventional in-person interviews. Instead, everyone whom the questionnaire was distributed was asked to fill it on their own to avoid any kind of disturbance or influence due to the presence of the people who carried out the survey. The square root of the population set our objective for the total number of respondents; since the population of the Municipality of Castro Verde is of 7603 people, ninety people were interviewed.

For the general public group (N = 36), participants were recruited among pedestrians and business establishments in the centre of Castro Verde village. The preferential targets were adults but some teenagers also participated in the survey.

In order to obtain as much people from the agricultural sector as possible the survey was performed during the period of the call to the Zonal Program of Castro Verde that was held in the “Campo Branco” Farmers’ Association. Contiguous to the office where the call was going on there is the store of the “Campo Branco” Farmers’ Association that sells several agrochemicals and veterinary medicinal products. Therefore people from both places were recruited (N = 31), that included, every kind of jobs related to agriculture and livestock farming, as well as land owners that may had other occupations not related to farming at all.

Servants from local authorities (N = 23) were recruited directly from their working places, namely the municipality hall of Castro Verde, and the parish offices at Castro Verde, Casével, Santa Bárbara de Padrões and São Marcos da Atabueira. Respondents were selected at random from different sections and working posts reaching people from different professional and educational backgrounds.

Results and discussion

The response rate was not measured but, except for local authorities, it can be estimated that only ca half of the people that were addressed accepted to fill the questionnaire. From our interpretation of people's excuses for not participating, it seems that they were afraid of being evaluated on they answered to the questionnaire and in many cases people had difficulties in reading and interpreting the survey.

Socio-demographics

The socio-demographic characterization of the respondents is given in table 5.I. Whereas for general population the proportion of men and women was almost the same, in the agricultural sector men were overrepresented (69 %), and in local authorities women were in larger number (65 %). People that accepted to participate among the pedestrians and business establishments were largely under forty years old (72 %). In the agricultural sector the majority lied in the range of 40-65 years old (55 %) but a significant percentage of people were under this range and older than 25 years old (35 %). Public servants from the municipality and parishes that participated were, of course in working age, mainly in the range of 30-50 years old (70 %). For all groups respondents inhabited mostly in Castro Verde village (parish), because except for the parish offices in the other villages, the survey was carried out there. People that lived in other municipalities were also considered for the survey because their professional activity was developed in the municipality of Castro Verde or because their municipality of residence was also included in the SPA.

Table 5.I. Socio-demographic characterization of the respondents. (Values are given in terms of percentage.)

	General public (n = 36)	Agriculture sector (n = 31)	Local authorities (n = 23)
Gender			
Male	47.2	69.0	34.8
Female	52.8	31.0	65.2
Age			
<18	13.9	0.0	0.0
19-24	8.3	6.9	4.3
25-30	13.9	20.7	17.4
31-40	36.1	13.8	26.1
41-50	11.1	31.0	43.5
51-65	13.9	24.1	8.7
>66	2.8	3.4	0.0
Education			
Illiterate	2.8	0.0	0.0
Basic – 1st degree	5.6	23.3	4.3
Basic – 2nd degree	8.3	6.7	4.3
Secondary – 1st degree	41.7	20.0	43.5
Secondary – 2nd degree	36.1	13.3	17.4
Academic degree	5.6	36.7	30.4
Occupation			
Student	16.7	0.0	0.0
Unemployed	5.6	0.0	0.0
Public servant	2.8	0.0	100.0
Farmer	2.8	53.3	0.0
Self-employed worker	11.1	16.7	0.0
Employee/ Worker	58.3	30.0	0.0
Retired	2.8	0.0	0.0
Municipality and Parish			
Castro Verde			
Castro Verde	81.8	62.1	60.9
Casével	0.0	6.9	8.7
Sta. Bárbara de Padrões	3.0	0.0	8.7
S. Marcos da Atabueira	0.0	3.4	4.3
Another Municipality	15.2	27.6	17.4

Most of the respondents from the general public group received 9 years (secondary first degree, 41 %) or even 12 years of education (secondary second degree, 36 %). One of the respondents in spite of being illiterate was able to read and fill the questionnaire with crosses. 58 % of the respondents were employed or working and this was the only group where students participated in the survey. The agricultural sector had the higher percentage of people with a university degree (37 %) but within this group the second highest level of education represented was the first degree (primary school) (23 %). This shows that this group consisted of two kinds of people: with higher education (engineers or veterinarians) that worked in the sector or were the land owners; and workers that performed tasks with a lower level of education (e.g. truck drivers or land workers) or that managed smaller agriculture fields. Respondents from local authorities were quite well educated, with 39 % of people with a university degree, but most of the servants had nine years of education (secondary first degree, 44 %).

The SPA

First of all, it was possible to know how respondents thought about the level of information they possessed on the SPA of Castro Verde (figure 5.1). On a scale of zero to five it was coincident that the mode for the responses was of three (followed by two). This does not clarify particularly the question as it is the medium level of the scale. But if we look at the extremes, zero and one for not informed at all, and four and five for very well informed, and the overall trend, a better outlook on the differences of opinions between groups is obtained.

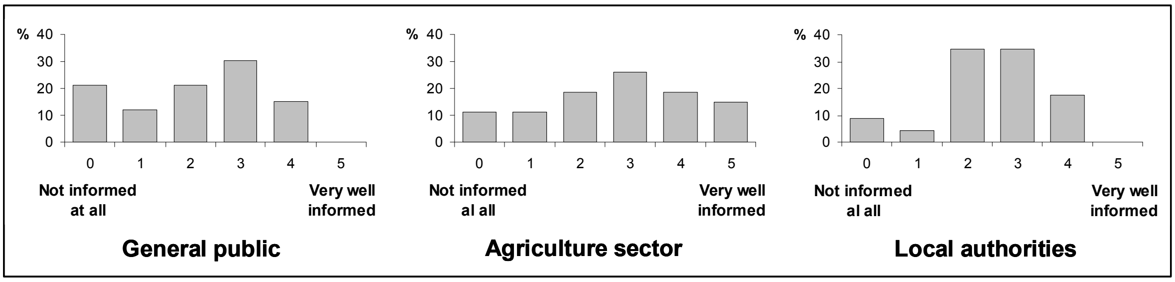

Figure 5.1. Respondents' opinion on the level of information they possess on the SPA of Castro Verde, from 0 as not informed at all to 5 as very well informed.

General public indicated the lowest level of information, with the highest percentage of respondents with reduced information level (33 %). Local authorities represented the group with least variability, indicating an average level of information, with just 13 % indicating a reduced level of information and no one assuming to be very well informed. The opposite was observed for the agriculture sector, where 22 % indicated a low level of information, while 33% considered themselves to be well or even very well informed. These results indicate that the group that dealt directly with the management of the SPA (agriculture sector), by carrying out directly their professional activities included a subgroup which considered itself to be knowledgeable in terms of information on the SPA characteristics.

In terms of precise aspects of the SPA the agricultural sector also presented a better insight as can be observed in the graphs of figures 5.2 and 5.3.

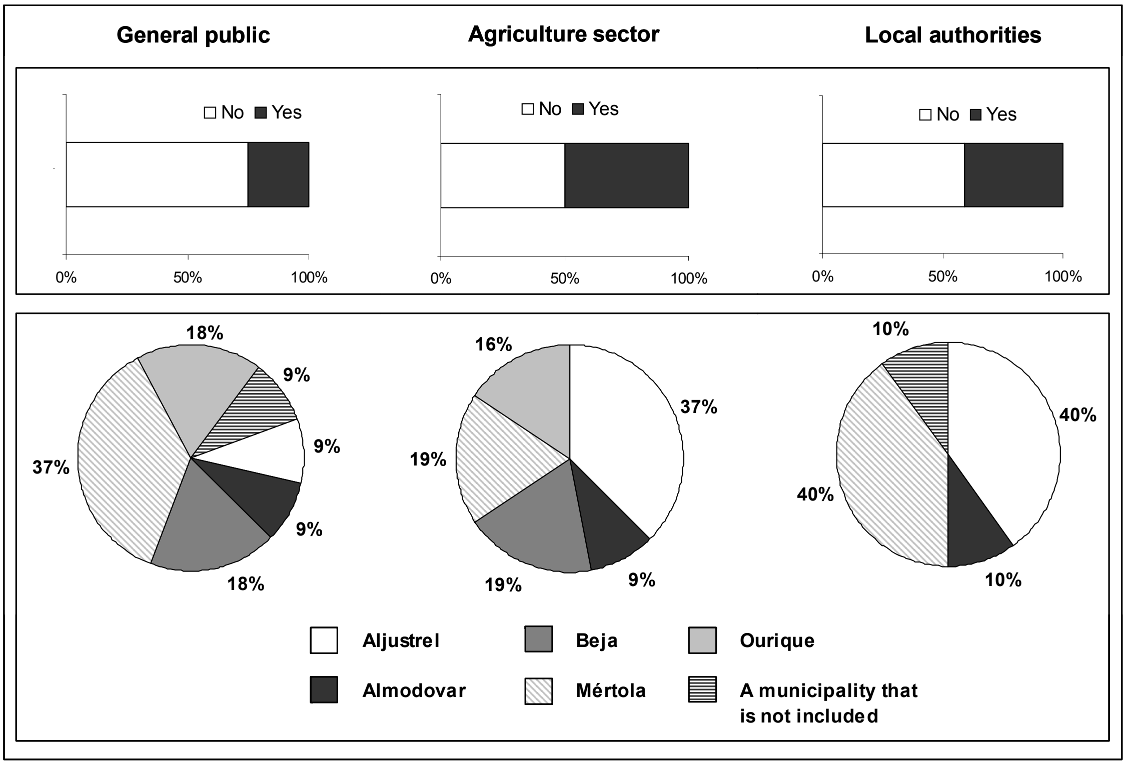

Figure 5.2. Besides Castro Verde, do the respondents know another municipality included in the SPA?

50 % of the respondents from the agriculture group knew at least one municipality included in the SPA, other than Castro Verde (figure 5.2). The municipalities also included are Aljustrel, Almodovar, Beja, Mértola, and Ourique, and all of them were referred by this group. From the general public, only 25 % new another municipality from the SPA, but 9 % of the respondents indicated one that is not included. 59 % of the public servants that participated new another municipality, but 10 % indicated a municipality that has not part in the SPA, and only two other municipalities (Almodovar e Mértola) were referred.

According to the Portuguese Institute for Nature Conservation [12] the SPA of Castro Verde in southern Portugal, Alentejo, is the most important Portuguese area for the conservation of steppe bird species such as the Great Bustard (*Otis tarda*) that is one of the most important labels of that region [15]. But there are also other species of conservation concern and that is why respondents were asked if they knew other birds besides the Great Bustard (figure 5.3). The majority of people from the agriculture and local authorities groups knew other protected species (respectively, 63 % and 61 %), namely the Stone Curlew (*Burhinus oedicnemus*), the White Strock (*Ciconia ciconia*), the Black-bellied Sandgrouse (*Pterocles orientalis*), the Common Crane (*Grus grus*), the Lesser Kestrel (*Falco naumanni*), the Woodchat (*Lanius* sp.), the Little Bustard (*Tetrax tetrax*), and the Montagu's Harrier (*Circus Pygargus*). From the general public, less than half of the respondents (46 %) stated to know other bird species. The species that were referred to more often in all groups were the Black-bellied Sandgrouse, the Lesser Kestrel and the Little Bustard. Among the local authorities' respondents, the Lesser Kestrel corresponded to 76 % of the references. This is not surprising since two months before the survey took place a book on the Lesser Kestrel was presented [16] with the cooperation of the Municipality of Castro Verde. This event may also have contributed to the fact that a larger number of public servants got to know an additional protected species from the SPA. Another curiosity is the fact that people from the agriculture sector indicated six bird species whereas the other groups referred to only five.

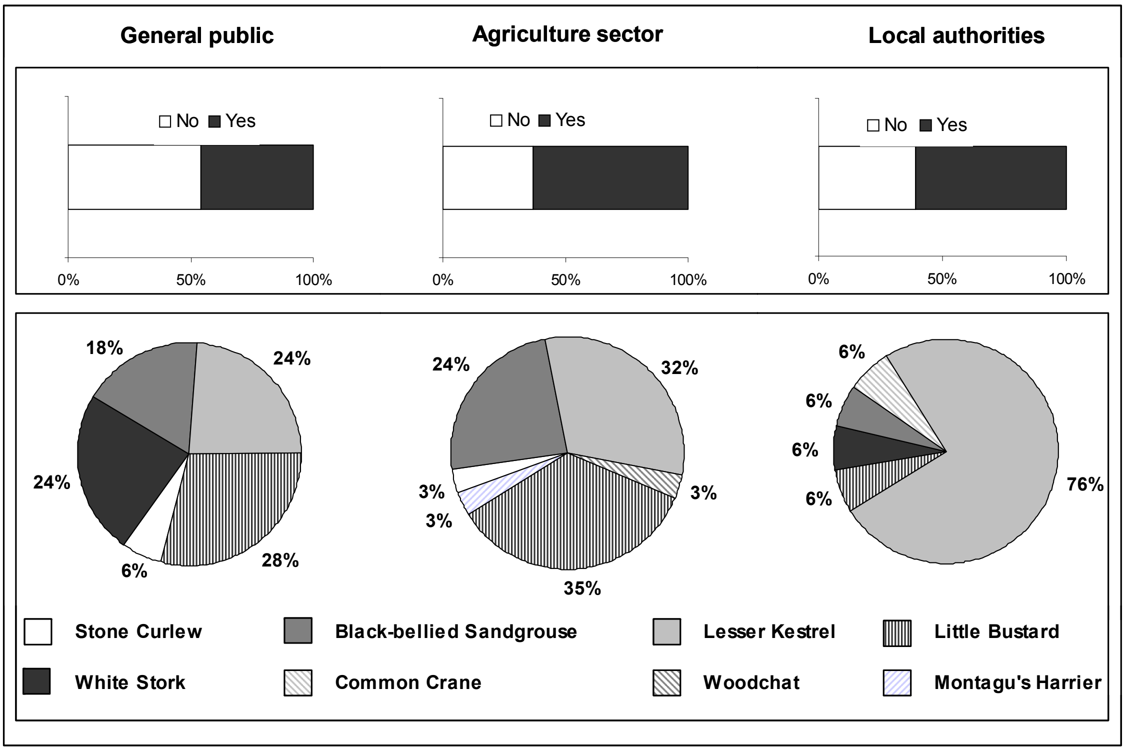

Figure 5.3. Besides the Great Bustard, do the respondents know another protected species in the SPA?

In a statement, how do people feel about the SPA? Is it an advantage or a disadvantage for the Municipality and the different human activities that may take place there? The majority of the respondents (90 % of the local authorities and 85 % of the general public) thought the SPA was a natural resource for educational, environmental and ecotourism purposes (figure 5.4). But a significant percentage of the agriculture sector (30 %) saw it as a surplus-value for agriculture and valorisation of the region, and 7 % considered the SPA a restriction for agriculture and local development. This sector experiences the direct consequences or any possible restrictions that may outcome from the classification of the site under the Natura 2000 Network. That is why the subject was a little bit more controversial, what makes it more surprising that 15 % – a higher value than the general public and this statement is not even considered by local authorities – of respondents claimed not to have an opinion on the subject.

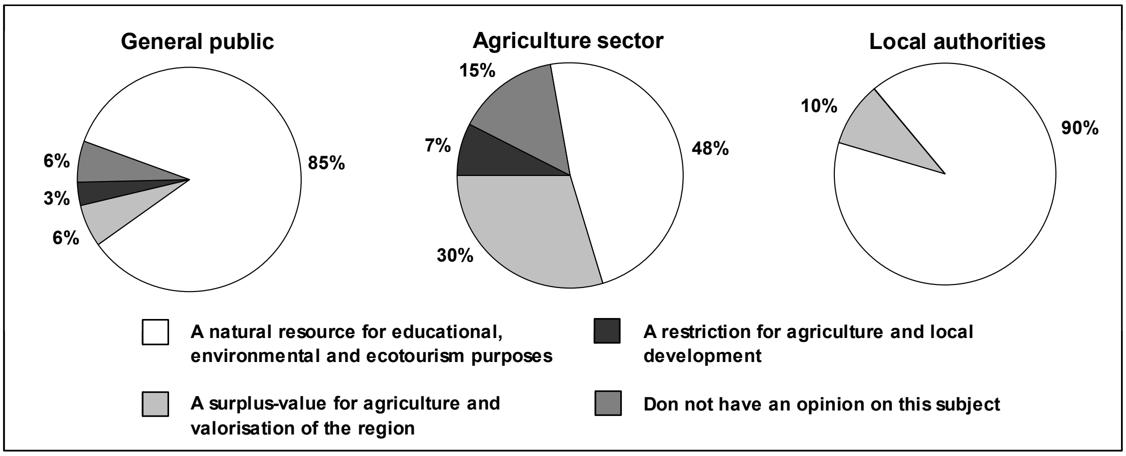

Figure 5.4. What do the respondents think of the SPA for Castro Verde and the other included municipalities?

The overall positive attitude towards the SPA among the inhabitants of the municipality of Castro Verde denotes a clear sensitivity towards the ecological value of this site, even to people that are directly affected from the management restrictions in a Natura 2000 Network. In a contingent valuation survey in Portugal, a significant part of the interviewed people (59.9 % web based and 45.7 % in-person) were willing to pay to preserve the Cereal Steppe o Castro Verde as an annual governmental tax and as a voluntary contribution) [17]. Both results show a considerable sensitivity of population in relation to services ecosystems and the environment may provide. By informing people about the SPA there is a large probability that an even greater understanding and perception of the ecological values will develop. But what are the sources of information from the Natura 2000 Network site of Castro Verde (figure 5.5)?

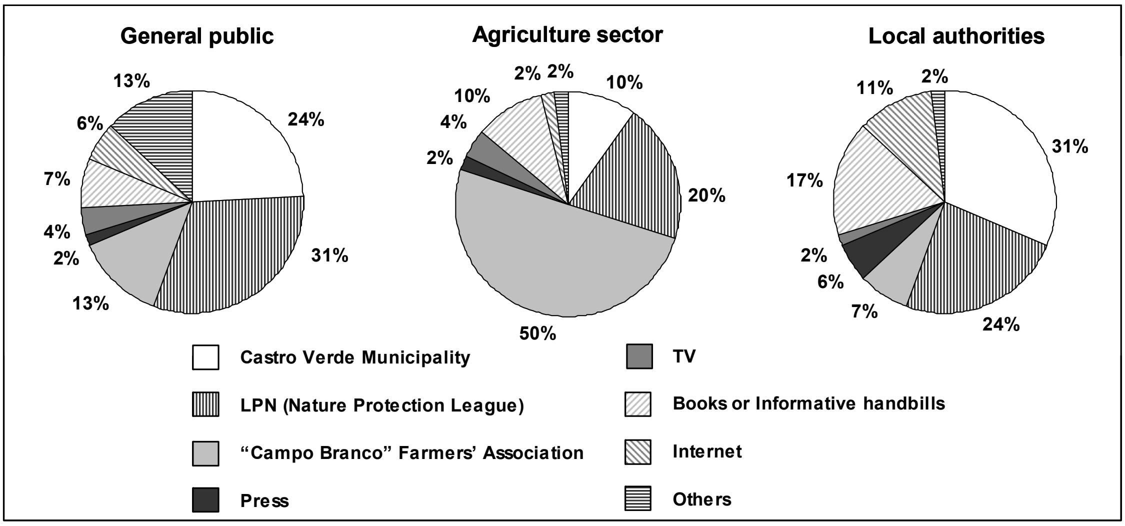

Figure 5.5. What was the source of information respondents have on the SPA for Castro Verde?

Not surprisingly, most of the respondents from the agriculture sector (50 %) stated their knowledge on the SPA of Castro Verde came from the “Campo Branco” Farmers’ Association and public servants (31 %) stated it was provided by the Municipality of Castro Verde. Looking at all target groups the environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information. This NGO has been developing in the region, since the early nineties, a sustainable model for agriculture, involving local farmers, and integrating it with several scientific, touristic and environmental projects. Press, television, internet and books or informative handbills, are also important sources of information to respondents.

SPA Conservation

The main habitat for steppic birds is created by extensive farming with fallow [18]. Thus the generality of species are favoured by open landfills. But how far do people understand that the maintenance of this habitat is important for the conservation of the SPA? Many may think that by planting trees, specially the autochthonous species like the Cork Oak or the Holm Oak a more favourable habitat to the generality of birds may be created. The majority of the respondents (87 %) from the agriculture sector and local authorities understood the importance of the maintenance of extensive agriculture, but almost half of the general public that participated in the survey did not know what the most important factor for the conservation of the SPA of Castro Verde was (table 5.II).

Table 5.II. Analysis of the respondents' opinion on the factors that underpin the conservation of the SPA of Castro Verde.

	General public (*n* = 36)	Agriculture sector (*n* = 31)	Local authorities (*n* = 23)
What is the most important factor for the conservation of the SPA of Castro Verde?			
Maintenance of extensive agriculture with cereal fields and fallow	44.4	86.7	87.0
Increase in Cork Oak and Holm Oak forest landscapes	13.9	3.3	4.3
Do not know	41.7	10.0	8.7
Do you think the traditional agricultural practices are only possible by being supported with agro-environmental funding (Zonal Program)?			
Yes	47.2	93.3	60.9
No	52.8	6.7	39.1

Since 1995 such agricultural scheme is being supported by agri-environmental measures' under the Zonal Program of Castro Verde. Reviewed in 2003 [19], this program allows financial compensation to farmers who voluntary agree to maintain the traditional agricultural system with the cereal-fallow rotation, in an area larger than one hectare. It is an important tool to overcome the fact that as a low intensity dry cereal farming land it represents a marginal economic system with a yield of 14 % of the EU average [18]. As can be observed from table 5.II the importance of the Zonal Program was absolutely clear for the agriculture sector (93 %), since the economic viability of their activity is dependent in the financial support. Whereas for local authorities the importance of this funding was also clear (61 %), but more than half of the general public (53 %) did not think so.

Risks to the SPA

The main threats for the SPA of Castro Verde and probable risks for the steppic bird species have been identified [15; 12; 18; 16]: intensification of agriculture and livestock farming – with increasing of agrochemicals input, and installation of fences and land irrigation systems – on one side, or abandonment of agriculture on the other; the forestation with Eucalyptus or Pine trees due to rather advantageous EC funding; and illegal hunting and death by electrocution when colliding with electric cables, especially to Great Bustard populations. Other less conspicuous inputs of toxic chemicals in the ecosystem are sewage sludge and livestock manure. In recent years in Castro Verde, wastewater sludge was used as fertilizer in a program aiming to prevent desertification and soil erosion. Previous studies under Mediterranean climatic conditions have showed the fertilization with sewage sludge may pose risk to soil invertebrates [20; 21]. As for livestock, toxic concentrations of veterinary medicinal products that can be found in dung and urine [22] may contaminate soil due to grazing or when manure is used as fertilizer in agriculture.

A final question was drawn in the survey so that respondents could reflect and assess a series of risks that might affect the SPA (figure 5.6). Albeit the fact that in table 5.II it was clear respondents perceived the importance of the maintenance of extensive farming, a reduced percentage of answers assessed the intensification of agriculture (and development of systems for land irrigation) with a high risk for the SPA. Many considered it as moderate risk, but this intermediate assessment was frequent in almost every factor that was presented. The issues that were considered with a higher risk by all target groups were agriculture abandonment, herbicides, illegal hunting and, at some extent, death by electrocution and the disposal of sewage sludge as soil amendment. The factors considered to pose a lower risk were the ones related to livestock – cattle increase and usage of manure as fertilizer in agriculture.

When comparing the assessments performed by the different target groups there were not any major differences. Still in some issues agriculture sector showed some small variations in the perception of risks. It was the group with a higher percentage of high risk assessments to intensification of agriculture (32 %, followed by the general public with 21 %), as the economy of this group has a direct relationship with the SPA activities it might be expected them to wish to turn farming as profitable as possible. However, the overall assessment must also consider their higher level of knowledge as well as the socioeconomic correction provided by the agro-environmental funding. Moreover, they looked to the risk of forestation with more careful, and 24 % of the respondents considered it as high. As for herbicides, they were looked at with less concern, when compared with the other target groups; 50 % of the respondents considered the risk of the utilization of herbicides as being high when this assessment was of more than 80 % in the other groups. There was also less concern towards illegal hunting (56 % high risk, 36 % moderate risk) in comparison with public and local authorities (ca 80 % high risk, ca 20 % moderate risk).

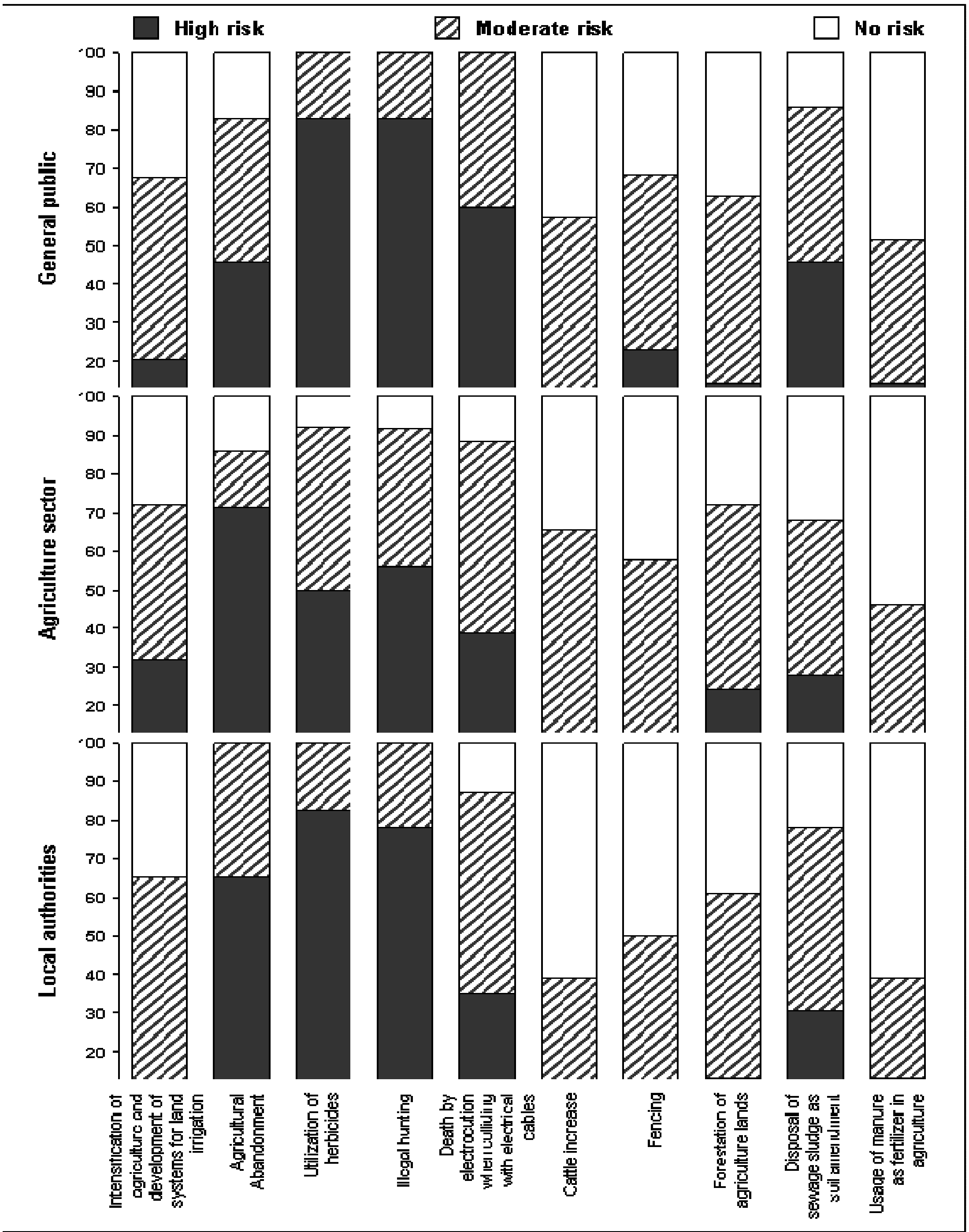

Figure 5.6. Respondents' opinion on risks affecting the SPA for Castro Verde.

Conclusion

Following a risk assessment in the SPA of Castro Verde and knowing the diverse perceptions and characteristics of the different stakeholder it is possible to develop tools for an effective risk communication framework, important in the environmental governance of this Natura 2000 Network site.

First of all in order to decide on the amount of information provided and how it is deployed, one must be aware of the cultural background of the population. And the only objective way of doing so is by the analysis of their educational background. The majority of the population has received at least more than 9 years of education (secondary first degree) and in the local authorities this proportion exceeds 90 %, having 30 % of the public servants an academic degree. The agriculture sector is the target group with a higher percentage of people that attended university (37 %) but a significant percentage of the group (23 %) has only been at primary school. Therefore the heterogeneity of the agriculture sector must be taken into account when preparing communication events.

The agriculture sector presented the better knowledge on precise aspects of the SPA – municipalities that are included in its limits and protected bird species – and a higher percentage of respondents considering themselves very well informed. Most of the people from general public and local authorities consider the SPA a natural resource for educational, environmental and ecotourism purposes but in the case of the agriculture sector it is also seen as a surplus-value for agriculture and valorisation of the region. The "Campo Branco" Farmers' Association and the Municipality of Castro Verde were considered the most important sources of information for, respectively, the agriculture sector and for public servants but the NGO, LPN, has shown to have an extremely relevant role in informing all target groups about the SPA of Castro Verde.

The importance of the maintenance of extensive agriculture of cereals with fallow rotation, supported by agri-environmental measures, is understood by local authorities and especially to the agriculture sector that relies economically in this scheme, but general public does not share this perception.

The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. They seem to disregard the intensification of agriculture, maybe because they do not link it, along with the development of systems for land irrigation, with the destruction of extensive cereal steppe habitats.

There are several potential factors that could contribute to this assessment such as knowledge, heritage, and socio-economy. The higher level of information on the environmental values of the SPA, perceived by the agriculture group and also confirmed by the answer to relevant questions, may indicate a greater capacity for perceiving certain risks, in particular the relationship between conservation and traditional extensive farming. Additionally, the cultural heritage, and the clear perception of agri-environmental funding as an essential need for maintaining sustainable agricultural practices, and last but not least, the fact that this funding has been available for years, should be considered. It is important to mention that only 7% of the enquired persons within this group perceived the SPA measures as limitations for agricultural development, while 30% perceived the opposite, considering the SPA as an opportunity for local valorisation. The Common Agriculture Policy of the EU is considered a key tool for socioeconomic balance among regions. The aims and objectives have been adapted and current measures focus mostly on the protection of the environment and the farmers' quality of life. In a global market, sustainable rural development is demonstrating a clear capacity as alternative to intensive agriculture. Issues such as food quality, food safety, food diversity, animal welfare, are more and more appreciated by the European citizens. The added value gained by these issues may compensate the final yield economic balance. Additional opportunities related to leisure activities such as ecotourism, and recognising the role of extensive agricultural in the SPA as a service provided by the farmers to the society (biodiversity conservation) that should be compensated, are also relevant when interpreting the differences in the perception among the three groups. Although the questionnaire does not allow a formal interpretation, a significant issue identified within this study is the role of source for information. The group related to the agricultural sector had received the information basically through an agricultural organization and an environmental NGO, the combined information seems to offer in general a proper level of knowledge on the ecological and socioeconomic implications of the SPA.

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Annex 5. Survey on the SPA of Castro Verde

We are undergoing a study in an area of high ecological value of Castro Verde designated as "SPA of Castro Verde", and we would like your help by answering the following survey questions in order to us to know how informed you are on this special protection area. This information will be taken into consideration in future actions.

THANK YOU VERY MUCH ON YOUR COOPERATION!

Gender: male ☐ female ☐

Age: _____

Education: ____

Occupation ____

Parish and Municipality of residence: ___________________________

SPA: SPECIAL PROTECTION AREA FOR WILD BIRDS

How informed are you about the SPA of Castro Verde? (from 0 as not informed at all to 5 as very well informed)

0	1	2	3	4	5

Besides Castro Verde, do you know another municipality included is this SPA?

YES		NO	

In case you said yes, indicate one: ________________________________

Do you know any protected species of this SPA besides the Great Bustard?

YES		NO	

Indicate one: __________________________

What do you think of the SPA for Castro Verde and the other included municipalities? (indicate one option only)

- A natural resource for educational, environmental and ecotourism purposes ☐
- A natural space with no advantage or usefulness at all ☐
- A surplus-value for agriculture and valorisation of the region ☐
- A restriction for agriculture and local development ☐
- Don not have an opinion on this subject ☐

What was the source of the information you actually have on the SPA of Castro Verde (indicate one or more options):

- Castro Verde municipality ☐
- LPN (Nature Protection League) ☐
- "Campo Branco" Farmers' Association ☐
- Press ☐
- TV ☐
- Books or Informative handbills ☐
- Internet ☐
- Others ☐
  Which ____

What is the most important factor for the conservation of the SPA of Castro Verde?

- Maintenace of extensive agriculture with ceral fields and fallow ☐
- Increase in cork oak and holm oak forest landscapes ☐
- No not know ☐

Do you think the traditional agricultural practices are only possible by being supported with agro-environmental funding (Zonal Program)?

YES		NO	

Assess the following agents according to your personal degree of acceptability towards the risk for the wild birds at the SPA of Castro Verde:

	HIGH RISK	MODERATE RISK	NO RISK
▪ Intensification of agriculture and development of systems for land irrigation			
▪ Agricultural Abandonment			
▪ Utilization of herbicides			
▪ Illegal hunting			
▪ Death by electrocution when colliding with electrical cables			
▪ Cattle increase			
▪ Fencing			
▪ Forestation of agriculture lands			
▪ Disposal of sewage sludge as soil amendment			
▪ Usage of manure as fertilizer in agriculture			

CHAPTER 6

Chapter 6. General Discussion

Risk Characterization

Given our hypothesis, due to the biomagnification of chemical pollutants along the food chain it would be expected the occurrence of secondary poisoning of protected birds in the Special Protection Area (SPA) of Castro Verde: Great Bustard (*Otis tarda*), Lesser Kestrel (*Falco naumanni*) and Montagu's Harrier (*Circus pygargus*). Hazard of herbicide glyphosate [1-3], and linear alkylbenzene sulphonate (LAS) [4-8] and cadmium [9-13] present in sewage sludge used for soil amendment had been identified in literature. Inclusively, in one hand secondary poisoning had been demonstrated previously [14-16] and contamination of birds due to metals [17; 18] and pesticides is well documented [19; 20]. On the other hand the Montagu's Harrier exhibits a positive mean population trend in Europe [21], and the populations of Great Bustard and Lesser Kestrel have been recently increasing in the SPA of Castro Verde [22; 23]. But one of the major threats to this SPA, and to high nature value farmlands in general across the EU, is the intensification of agriculture [24; 25] with deleterious consequences to biodiversity, namely birds [26]. Therefore assessing real- and worst-case scenarios for the exposure of toxic chemicals will give us a predictive in-sight on agriculture intensification and also on the repeated exposure effects to bird communities due to present extensive agricultural practises.

For the study of the interactions between trophic levels and determining the transfer of chemicals through the trophic chain terrestrial microcosms were used, and predicted environmental concentrations (PECs) and bioaccumulation factors (BAFs) were obtained. Experimental data allowed us to calculate values for plant and invertebrate species important in the ecological receptors' food chain. Plant species important to the agricultural scheme of the SPA of Castro Verde were chosen in accordance to *O. tarda* feeding preferences: common wheat (*Triticum aestivum*), chickpea (*Cicer arietinum*), and cultivated radish (*Raphanus sativus*) [27-29]. Earthworms *Eisenia andrei*, were tested as key elements involved in secondary poisoning of organisms that feed upon them [13], and due to the role they play in water infiltration and storage and soil aeration [30] thus contributing for the mobilization of metals [31]. Locusts *Schistocerca gregaria* were selected since Orthoptera are important food items for juvenile *O. tarda* [28] but also to *F. naumanni* [32] and, to a less extent, to *C. pygargus* [33]. BAF and PEC values for small mammals and PEC values for target bird species were calculated adapting the formulas from the Guidance Document on Risk Assessment for Birds and Mammals under the plant protection products' directive [34]. Predicted no effect concentrations were derived from literature toxicity data following the principles of the Technical Guidance Document on Risk Assessment [35]. An additional safety factor of 10 was included to cover for the individual variability within bird species, as the assessment focuses on defined species with ecological value which should be protected at least at the population level within the area. For transparency reasons this factor was not included in the PNEC derivation, but it was used in the interpretation of the risk values. Finally, for the characterization of risk a standardized approach for all chemicals was performed on the simplified comparison of the PEC/PNEC ratio. The refinement of risk assessment was done using the probabilistic approach with Monte Carlo analysis (10000 trials) performed with Crystal Ball software [36]. The characterization of risk with the probabilistic approach allowed us to distinguish four classes of risk: (i) PEC/PNEC < 0.1, very low risk; (ii) 0.1 < PEC/PNEC < 1, low risk; (iii) 1 < PEC/PNEC < 10, potential risk; and (iv) PEC/PNEC > 10, risk. Calculations for probabilistic risk assessment under a realistic scenario are presented in table 6.I.

Table 6.I. Probabilistic assessment of risks posed to birds of conservationist concern from the SPA of Castro Verde under a real-case scenario.

	Herbicide	**Sewage Sludge**	
	Glyphosate	**Cd**	**LAS**
Adult Great Bustard	100 % low risk	100 % low risk	100 % potential risk
Juvenile Great Bustard	52 % low risk 48 % potential risk	39 % low risk 61 % potential risk	100 % potential risk
Lesser Kestrel	100 % low risk	93 % low risk 7 % potential risk	100 % potential risk
Montagu's Harrier	79 % very low risk 21 % low risk	1 % low risk 99 % potential risk	100 % potential risk

Herbicide usage

The uptake of glyphosate that resulted from agricultural application rate could only be determined by analytical methods for plants and therefore PEC was calculated only in the case of adult Great Bustard and Montagu's Harrier. However, for the assessment of glyphosate its degradation metabolite AMPA must also be taken into account and consequently when comparing the risk of the two compounds for the same bird, the higher level of risk must be considered. The overall risk for Montagu's Harrier is very low, with a 20.94 % probability of low risk due to AMPA. The pathway for herbicide in *C. pygargus* food chain is affected by the low BAF values of small rodents (herbivorous mammals), the most important item of its diet, thus explaining the low uptake and concomitant low risk. The risk of glyphosate may be considered to be low for the adult Great Bustard. Considering the unmetabolized parent glyphosate, there was no exposure, i.e. no contact between stressor and receptor, for juvenile Great Bustard and Lesser Kestrel since it was not bioaccumulated in their food items, i.e. locusts. But the breakdown product AMPA was accumulated along the food chain though posing low risk to *F. naumanni* but ca. 50 % of potential risk to juvenile *O. tarda* despite being indicated as a low toxic substance to vertebrates [2]. The effects on juvenile individuals have consequences at the turnover of the population thus jeopardizing the conservation of the species.

Sewage sludge amendment

The risk from Cd and LAS present in sludge-amended soils must be assessed in separate scenarios since when sewage sludge was added to soil in Castro Verde, only metals were analysed in accordance to the Sewage Sludge Directive 86/278/EEC [37] (and national legislation, n.º 118/2006 [38]).

In the case of Cd, several scenarios were considered but microcosm experiments were performed for a worst-case considering the concentration in sewage sludge; hence BAF calculations were made with PEC in soil from this scenario. A more realistic scenario was assessed with the estimated soil concentration dependent exclusively from sewage sludge amendment but the real-case scenario comes from adding to this PEC the Cd from baseline concentrations in agricultural soil from Castro Verde, that exactly matches the PEC for a generic Regional environment (PECregional) calculated in the Risk Assessment Report [13]. In this scenario risks from Cd are low for adult Great Bustard, but there is a 7 % probability for Lesser Kestrel, a 61 % probability for juvenile Great Bustard and even a 99 % probability for Montagu's Harrier, of potential risk from the amendment of sewage sludge. Thus the top predator *C. pygargus* has the most critical food chain for secondary poisoning. Another scenario of concern was the one of the maximum level of Cd permitted by the Sewage Sludge Directive 86/278/EEC [37] (and national legislation, n.º 118/2006 [38]) to be added to agricultural land per year, that even disregarding the baseline Cd concentration showed a potential risk to Montagu's Harrier of 56 %.

PEC for LAS was derived from soil concentrations in Denmark in a worst-case scenario with a sludge dosage of 2 T ha^{-1}. But according to the information regarding the usage of sludge in Castro Verde, a mass of 5-6 T ha^{-1} at depth of 30-75 cm was amended [39], probably turning the worst-case Danish PEC in a realistic exposure for our case-study. There is a common agreement on the fact that LAS has no risk for terrestrial (and aquatic) compartment as assessed by HERA [8] and the occurrence of bioconcentration is highly unlikely with an extremely low potential for secondary poisoning [7]. Nonetheless the present experimental work with terrestrial microcosms and respective risk assessment indicate that LAS may bioaccumulate in plants and concentrate through the food chain, and be responsible for secondary poisoning, having a potential risk for the considered bird target species, as assumed in our initial hypothesis. The fact that the soil from Castro Verde has low organic matter content may account for plants and invertebrates uptake hence influencing its transfer along the food chain.

Risk Communication

The perception of the ecological values and risks from extensive agriculture to different stakeholders – general public, agriculture sector and local authorities –, in the SPA of Castro Verde was assessed with a questionnaire-based survey, as part of the risk communication process. The agriculture sector presented the better knowledge on precise aspects of the SPA and a higher percentage of respondents considering themselves very well informed. The generality of respondents from all target groups were more sensitive to risks posed to the SPA by agriculture abandonment, herbicides, illegal hunting and also to death of birds by electrocution when colliding with electric cables and the usage of sewage sludge as soil amendment. They seem to disregard the intensification of agriculture, maybe because they do not link it, along with the development of systems for land irrigation, with the destruction of extensive cereal steppe habitats and the input of chemical toxicants. The environmental non-governmental organisation (NGO), LPN (Nature Protection League) seems to be an important source of information about the SPA to the people from the region.

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